

FORMATION OF IRON COMPLEXES IN SOIL ORGANIC MATTER AND THEIR
INFLUENCE ON MOBILITY AND BIOAVAILABILITY OF ANTIMONY

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FORMATION OF IRON COMPLEXES IN NATURAL ORGANIC MATTER AND THEIR INFLUENCE ON MOBILITY AND BIOAVAILABILITY OF ANTIMONY

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Antimony (Sb) is a metalloid belonging to group 15 of the periodic table. Chemical similarities between arsenic (As) and Sb produce concerns about potential health effects of Sb and enrichment in the Environment.

Sb is found in the environment as an oxyanionic species, antimonate (Sb(OH)_6^-). As a result of its net negative charge, antimonate was not initially predicted to have strong interactions with natural organic matter.

It has been suggested that oxyanionic species could bind the negatively charged organic matter via a ternary complexation mechanism, in which cationic metals mediate the strong association between organic matter functional groups and oxyanions.

The structure of these complexes remains poorly characterized. XANES spectroscopy was performed on organic soils amended with increasing iron levels in order to elucidate the structure of organically complexed iron. Humic acid complexes of iron were also synthesized and examined using XANES and Mossbauer spectroscopy. Two distinct iron sites were found in organic materials. A monomeric iron site and an oligomeric site consisting of small clusters of iron at sub-oxide levels.

Phosphate exchangeable Sb was predicted to represent the majority of soil

bound Sb. However, phosphate extractable Sb from soils is lower than anticipated. The affect to phosphate on Sb retention in organic soils was examined. Phosphate addition significantly reduced Sb retention in organic soils treated with Fe.

The influence of organically complexed Fe on the mobility of Sb was assessed. Increasing Fe amendments resulted in an increase in Sb retention in organic soils. Further examination of the bioavailability of Sb to maize seedlings as a function of organically complexed Fe was examined using a greenhouse study. An unexpected increase in plant tissue Sb was observed as organically complexed Fe increased, which was not predicted by extractions commonly used to assess bioavailable Sb.

Extraction of soils with organic acids common to the maize rhizosphere suggested that organic acid exudation can readily mobilize Sb bound by organic iron complexes.

Overall, iron complexes in soil organic materials were found to have significant implications on mobility and bioavailability of Sb. Additionally, methods used to assess bioavailable Sb underestimate Sb mobility in organic soils.

BIOGRAPHICAL SKETCH

Corey graduated magna cum laude from SUNY Fredonia with a Bachelor of Science degree in Environmental Sciences in May of 2006. In August of 2006 he began his graduate studies in the Field of Environmental Toxicology at Cornell University, under the guidance of Dr. Murray McBride.

Dedicated to my family and friends, because they never had any doubts.

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CHAPTER 1

ENVIRONMENTAL BEHAVIOR AND TOXICOLOGY OF ANTIMONY

1.1 Occurrence, Exposure and Toxicity

1.1.1 Use and Environmental Occurrence

Antimony (Sb) is a metalloid belonging to group 15 of the periodic table. Chemical similarities between arsenic (As) and Sb produce concerns about potential health effects of Sb and enrichment in the Environment. The chemical behavior of Sb in the environment is often assumed to be similar to that of As (Casiot et al., 2007). However, significantly less is known about the toxicity and environmental chemistry of Sb (Filella et al., 2002a).

Antimony occurs naturally as a trace element in the soil as a result of the weathering of several Sb containing minerals, primarily stibnite (Sb_2S_3) and valentinite (Sb_2O_3), (Filella et al., 2002a). Background Sb concentrations in soils have been reported between .02 – 7.01 mg kg⁻¹ depending on parent material and specific soil type (Chen et al., 2001, Kabata-Pendias and Pendias, 1984).

Sb has been used commercially for centuries but has increased in recent years, leading to an increase in Sb concentrations detected in the environment. Ancient Greeks and Romans used the antimony mineral stibnite, called stibium, as an eyebrow paint. Nowadays, Sb has many industrial uses, with an estimated mine output of 169,000 metric tons mined globally in 2011 (U.S Geological Survey, 2012). Primary industrial uses of Sb include use of antimony trioxide (Sb_2O_3) as a flame retardant in textiles, plastics and electronic devices as well as use of antimony as a hardening

agent in metal alloys and use in semiconductors, paint pigments, ceramics and glass (Filella et al., 2002a). Antimony triacetate is also used as a catalyst in the production of PET plastics (Cheng et al., 2010). Thus, textiles, plastics and electronic wastes become significant sources of Sb to the environment, particularly from municipal waste incineration (Paoletti et al., 2001).

Bullet casings have been found to contain up to 7% Sb by mass (Rooney et al., 1999). This has led to increased concentrations of Sb in shooting range soils as a result of weathering of bullets and bullet casings. Concentrations in shooting ranges have been reported between 35 and 17500 mg kg⁻¹ Sb (Johnson et al., 2005, Scheinhost et al., 2006, Robinson et al., 2008). Johnson et al. (2005) report that Sb derived from bullet weathering is more mobile than lead under standard soil conditions and thus surface waters near shooting ranges have been found to contain elevated levels of Sb.

Antimony contamination along roadsides has been found as a result of abrasion dusts from antimony-containing brakes pads. Furata et al. (2005) report a mean release of 1.24 grams Sb per car per year in Japan. Sb concentration along roadsides was strongly correlated with traffic patterns and distance from the road with the greatest antimony concentrations reaching 8.68 mg kg⁻¹ immediately adjacent to busy roads and decreasing with distance (Ceriotti and Amarasiriwardena 2009, Amereih et al., 2005). This suggests that cars could potentially become a significant source of antimony in urban environments.

The majority of Sb contamination results from mining and industrial emissions from smelting (Ashley et al., 2007, Wilson et al., 2010, Telford et al., 2009). Soil

antimony concentrations in smelting regions have been reported as high as 80,200 mg kg⁻¹ (Wilson et al., 2004). Antimony concentrations reported in peat and ice cores in non industrial areas as a result of long range atmospheric transport provide a history of increasing industrial use of antimony and subsequent release to the environment (Shotyk et al., 1996, Krachler et al., 2005).

1.1.2 Exposure

A shopping basket study of antimony concentrations in bottled water show concentrations below the tolerable daily intake level set by the World Health Organization (Well et al., 2011, WHO 2012). However, bottled water at elevated temperatures has been shown to contain Sb concentrations that exceed the maximum concentration level of 6 ppb (Westeroff et al., 2008).

Work by Andra et al. (2012) suggests that the presence of dissolved carbon dioxide in carbonated consumer products coupled to additions of flavors and color to noncarbonated products could explain the elevated leaching of Br and Sb. This is confirmed by antimony concentrations in commercial juices, which have been found to be above the EU limit for drinking water by up to a factor of 2.7 (Hansen et al., 2011). It has also been suggested that PET microwave food containers could leach high levels of Sb into food (Well et al., 2011).

Acute Sb poisoning of humans or animals via ingestion of Sb-contaminated soil or consumption of plants grown on Sb-contaminated soil has not been reported. Assuming an average Sb concentration in vegetables grown in a contaminated garden of 100 mg kg⁻¹ dry weight, a value that has rarely been found exceeded under field conditions, then a person hypothetically would have to consume in average 1 kg dry

matter of these plants per day in order to reach a dose of 100 mg day⁻¹ Sb, i.e. the threshold of Sb intake considered to be critical by the world health organization (Bowen 1979). However, accumulation of Sb in plants have not necessarily been associated with decreased yield (Tschann et al., 2009) This could mean that elevated Sb levels could occur in vegetables, acting as a potential route of exposure.

1.2 Toxicity and Metabolism

Antimony is not an essential element in plants or animals (Adriano, 2001). It is considered acutely toxic and it can induce chronic health effects (WHO, 2003; EPA, 2000). The toxicity of antimony was originally thought to be similar to that of arsenic because of its close proximity in the period table and the resulting similarities in chemical characteristics. For instance, the trivalent state of antimony is more toxic than the pentavalent state (Thanabalasingam and Pickering, 1990; Smichowski et al., 1998). In the absence of adequate experimental data for Sb, it has been common practice to assume that Sb behaves similar to arsenic because of the chemical similarities of the two elements. However, several key differences exist between arsenic and antimony behavior in the body, the most notable being that antimony is not methylated in vivo and is readily excreted in the bile and the urine (Baily et. al., 1991). Antimony in the body does elicit a toxic response despite its relatively short residence time.

Despite the use of sodium stibogluconate ($\text{Sb}_2\text{C}_{22}\text{H}_{38}\text{Na}_3\text{O}_{26}$) as a treatment for Leishmaniasis for the last 75 years, not much is known about the effect of inorganic Sb on the metabolism of different organisms. It is known that the Sb (V) contained in drugs used against Leishmaniasis depend on the reduction of Sb (V) to Sb (III) in the

liver to reach their full effectiveness against the parasite (Mishra et al., 2007).

Sb (III) and As (III) are known to form strong bonds with functional groups such as the thiolates of cysteine residues. Like Sb(III), also Sb(V) prefers SH to OH groups, but binds to both.

When they react with these groups in proteins, they frequently inhibit catalytic or biological activity (Bhattacharjee and Rosen 1996, Rosen 2002). This may lead then to oxidative stress when enzymes in oxygen metabolism are affected. This effect is known for various heavy metals that are not directly involved in oxygen metabolism. One may theorize that also high concentrations of Sb may damage tissues in this way (Schutzendubel and Polle 2002). Patients receiving pentavalent antimony agents during treatment of leishmaniasis often experience pancreatitis, which subsides, when therapy is stopped (Gasser et al., 1994). Other symptoms observed after exposure to pentavalent antimony include renal tubular acidosis (Horber et al., 1993), and impairment of feeding habits and retardation of weight gain in rats as well as a dose-related reduction in hemoglobin concentration and hematocrit. Red cell count was also reduced in treated rats as well as a significant increase in the white cell count (alKhawajah et. al., 1997) Gustafsson et al. (1987) also found that patients treated with pentavalent antimony for kala-azar experienced an interference with cardiac, liver and renal function.

The activities of lysosomal glycohydrolases were determined in order to evaluate the asymptomatic toxic effects of low levels of exposure to arsenic and antimony in art glass workers by a fluorimetric assay. Secretion of lysosomal glycohydrolases was increased by antimony at a concentration of $200 \mu\text{g L}^{-1}$ (Goi et al., 2003).

Urinary antimony, cadmium, cobalt, and tungsten may be associated with chronic cerebrovascular Disease (Agarwal 2011). In an investigation of the trace element content in myocardial and muscular biopsy samples with clinical, haemodynamic, and histological diagnosis of idiopathic dilated cardiomyopathy (IDCM), there was a large increase ($>10,000$ times for mercury and antimony) in myocardial specimens (Frustaci et al., 1999).

The concentrations of the immunoglobulin (Ig) G subclasses, IgE, interleukin-2, interferon-gamma, and interleukin-4 in sera obtained from workers exposed to antimony through antimony trioxide manufacture were determined and compared with those of control subjects (Kim et al 1999). The serum levels of IgG1, an Ig involved in host defense against many microbial infections, were significantly lower in the sera of Sb-exposed individuals than in the controls. The serum concentrations of IgE, an Ig mediating allergic hypersensitivity, were also lower in the Sb-exposed workers than in the controls. The levels of interleukin-2 and interferon-gamma, multifunctional cytokines for T-cell-mediated immunity, were lowered in the factory workers (Kim et al 1999). A significant positive correlation between IgG4 and urine Sb levels was found among the Sb-exposed workers, indicating a possible role of IgG4 in Sb-mediated pulmonary or skin pathogenesis (Kim et al 1999).

1.2.2 Genotoxicity and Carcinogenicity

Both trivalent and pentavalent antimony compounds are generally negative in non-mammalian genotoxicity tests, while mammalian test systems usually give positive results for Sb(III) and negative results for Sb(V) compounds (De Boeck et al., 2003). Antimony trioxide treatment is associated with increased apoptosis associated with

induced reactive oxygen species (ROS) as well as differentiation markers. When the buffering capacity of the cell is decreased by depleting glutathione, ROS production and apoptosis is enhanced (Mann et al., 2006). Although apoptosis was not found in cells immediately following 4-h SbCl_3 treatment, DNA fragmentation was detected in CHO-K1 cells after a 4 hour SbCl_3 treatment and for 16 hours post-incubation (Huang et al., 1998)

A 4 h exposure to $>50 \mu\text{M}$ antimony trichloride (SbCl_3) could induce micronuclei formation in cultured Chinese hamster ovary (CHO-K1) cells, human bronchial epithelial (BES-6) cells, and human fibroblasts (Huang et al., 1998). Chromosomal mutagenicity induced by arsenic (III) was significantly suppressed by antimony (III), suggesting the necessity to identify putative environmental co-contaminations of antimony in the regions contaminated with arsenic and to determine the impact of antimony coexposure on arsenic genotoxicity and carcinogenicity in man in vivo (Gebel et al, 1997). Antimony (III) oxide by inhalation has been shown to cause lung cancer in female rats (Gebel et al., 1997).

Trivalent antimony is thought to cause significant toxicity due to its high affinity for sulfhydryl groups in biological macromolecules and several studies suggest that inhalation of trivalent forms of antimony can result in various precursors of cancer (Groth et al.,1986), Watt (1983). However, the EPA found that neither study was adequate to “reasonably determine or predict the oncogenic risk to humans exposed to these substances.” A more recent study compared the toxicity of trivalent arsenic to antimony (III). In comparison to trivalent arsenic, trivalent antimony proved to be five times less cytotoxic and one order of magnitude less genotoxic. This has

been attributed to the ability of As (III) to generate DNA-protein crosslinks and DNA strand lesions as two independent processes. In contrast, Sb(III) induced DNA strand lesions but not DNA-protein crosslinks (Gebel et al., 1997). Sb(III) has also been shown to inhibit DNA repair of double strand breaks that may be induced by radiation or other damaging events (Takahashi et al., 2002)

1.2.3 Environmental toxicology

Only a few toxicity tests have been performed with animals, plants or other organisms exposed to Sb contaminated soil. Aqueous extracts of a spiked Luvisol and Chernozem that had been spiked with Sb₂S₃ and Sb₂S₅ (aged for 6 months) were toxic to the soil alga *Chlorococcum infusionum* (Hammel et al., 1998). Kuperman et al., (2006) determined EC₅₀ values for the reproduction of worms and collembola in Sb₂(SO₄)₃ spiked soils. They obtained 70 mg kg⁻¹ for cocoon production of the earthworm *Eisenia fetida*, 316 mg kg⁻¹ and 169 mg kg⁻¹ for juvenile production of the enchytraeid *Enchytraeus crypticus* and the collembolan *Folsomia candida*, respectively.

The toxic effects of trace elements are usually most severe when they are present as free ions. Therefore for example in plants, most trace elements found in the tissues are complexed with organic ligands (Hall 2002), such as low molecular weight organic acids, plants produce two classes of metal-binding polypeptides: phytochelatins and metallothioneins (Cobbett and Goldsbrough, 2002). Maeda and Ohki (1998) detected metallothionein-like polypeptides in algae of the species *Chlorella vulgaris* exposed to Sb (III).

Plants were generally found to be more tolerant to soil Sb than soil fauna. Oorts et

al. (2008) determined the EC_{50} values of total and soluble soil Sb concentrations for plant growth. They spiked soil with up to $10,000 \text{ mg kg}^{-1} \text{ Sb}_2\text{O}_3$ and found mainly Sb V, around 70%, in soil solutions, extracted by centrifugation. They found a 50% reduction of root elongation for barley at $39 \text{ mg L}^{-1} \text{ Sb}$ in soil solution and a 50% shoot yield reduction for lettuce at $41 \text{ mg L}^{-1} \text{ Sb}$ in solution. Oorts et al., (2008) found 50% reduction of root elongation in barley and 50% reduction of shoot growth in lettuce at $\sim 40 \text{ mg L}^{-1} \text{ Sb}$ in centrifuged soil solution after spiking the soil used in their study with $\sim 7 \text{ g kg}^{-1} \text{ Sb}$ in form of Sb_2O_3 at the time of sampling, $\sim 70\%$ of the Sb in solution was present as Sb(V). He (2007) found no significant difference in the toxicity of Sb (III) and Sb(V) on root and shoot growth of rice grown in pots.

Davis et al. (1978) showed that phytotoxicity of Sb does not necessarily require Sb translocation to shoots. Growth of barley (*Hordeum vulgare*) was depressed in sand cultures at concentrations of $50\text{-}100 \text{ mg L}^{-1} \text{ Sb}$ in solution, although Sb was below the detection limit in the shoots ($< 2 \text{ mg kg}^{-1}$). Phytochelatins, which are rapidly produced in plants under heavy metal stress (Rauser 1995, Zenk 1996), were shown to contribute to Sb tolerance in yeast and wheat (Wysocki et al., 2003).

1.3 Biogeochemistry of Antimony

1.3.1 Speciation

Antimony has an s^2p^3 outer orbital electron configuration, which provides a range of oxidation states between -3 and +5, the most common oxidation states being +3 (antimonite) in anoxic environments and +5 (antimonite) in oxic environments. This results in Sb being found in the environment as an oxide, hydroxide or oxyanion, with antimonite occurring as a neutrally charged species Sb(OH)_3 in reducing conditions

and antimonite occurring as an oxyanionic species with a net -1 charge Sb(OH)_6^- in oxic environments. Both Sb(III) and Sb(V) have been found in oxic conditions due to photochemical reduction (Cutter and Cutter, 2005), slow reduction kinetics (Filella et al., 2002a), and stabilization by organic ligands (Sun et al., 1982). Additionally, complexation of antimony by sulfur have been shown to be favorable under reducing conditions and precipitation of antimony containing minerals has been observed (Takayanagi and Cossa, 1997; Vink 1996)

Data on organic antimony species is limited, particularly in soils. However methyl antimony species have been reported in aquatic environments under both oxic and reducing conditions (Andreae et al., 1981; Jenkins et al., 1998).

This speciation determines much of the environmental behavior of Sb much like it does for arsenic. However, where the structure of antimonite is analogous to that of arsenite, antimonate is octahedrally coordinated compared to the tetrahedral coordination of arsenate or phosphate, which accounts for differences between behavior of Sb and As in terms of environmental chemistry.

1.3.2 Antimony in soil environments

Differences in soil retention of Sb can be attributed to the octahedral coordination and net negative charge of the antimonite oxyanion (Sb(OH)_6^-), compared to the neutral antimonite (Sb(OH)_3). The negative charge of antimonate would suggest greater retention at lower pHs as anion exchange sites become more significant. Indeed, a solubility minimum has been observed for antimonate at pH 4 in whole soil experiments (Ashley et al., 2003). Antimonite showed consistent retention (80-100%) over a wide pH range (pH 1-12).

King et al (1988) reported a greater retention of antimony by mineral soils when compared to organic soils and retention of Sb was found to increase as the clay and oxide mineral fractions increased (Gal et al., 2006). In contrast, Tighe et al., (2005b) reported greater than 80% retention of antimony by acidic organic soils.

1.3.3 Interactions with silicate minerals

The influence of alumino-silicate minerals on antimony retention in soils remains unclear. It is likely that non-specific anion exchange could account for antimonate retention but is unlikely for antimonite (Gal et al., 2006). These mechanisms would depend greatly on pH and the point of zero charge for the minerals present (Matera and Le Hecho, 2001). However, exchangeable antimony, as determined by extractions with 1M KCl remains low, with 57-99% of Sb remaining un-exchangeable, suggesting more specific adsorption mechanisms for Sb retention (King 1988).

1.3.4 Interactions with Oxides and hydroxides

Oxides of manganese, iron and aluminum have been shown to be key factors in antimony mobility (Gleyzes et al., 2002; Manaka, 2005). Antimony (III) was shown to have the greatest adsorption to MnOOH with decreased interaction with Al(OH)₃ and FeOOH respectively. However, due to the relative rarity of MnOOH in soils, Al(OH)₃ and FeOOH are more likely to control mobility of Sb(III) in soils (Casiot et al., 2007). These interactions were found to be pH dependent with the greatest sorption (<80%) occurring below pH 6 (Thanabalasingam and Pickering, 1990). Leuz et al., (2006b) showed stronger sorption of antimonite than arsenite on goethite; the authors suggest a similar mechanism for Sb and As binding by the formation of bidendate, inner sphere corner sharing complexes.

Interactions of Sb(V) with oxides of manganese and aluminum remain unclear. However, iron oxides have been shown to be very important sorbants of Sb(V). These interactions have been shown to be more pH dependent than sorption of Sb(III) to Fe oxides (Leuz et al., 2006). In contrast to Sb(III), antimonate was shown to sorb less strongly on goethite than arsenate (Leuz et al., 2006) or phosphate (Nakamuru and Sekine, 2008). Mixed inner sphere complex formation has been suggested for the specific adsorption of Sb(V) on Goethite (Scheinost et al., 2006). Under acidic conditions, Sb(V) has also been shown to have a strong interaction with hematite, with very slow dissolution kinetics (Ambe, 1987). This binding was shown to be consistent with binding by a combination of surface sites (Pierce and Moore, 1982). Amorphous iron oxides show a similar adsorption maxima as hematite and goethite, with 95% of Sb(V) being sorbed at pH 4, suggesting a similar specific adsorption mechanism involving a mixture of inner sphere surface complexes (Tighe and Lockwood, 2007).

1.3.5 Interactions with soil organic matter

The importance of soil organic matter on Sb retention has been underestimated until relatively recently. The low retentions observed for As were assumed to apply to antimony and thus Sb interactions with organic materials received less attention (Lintschiger et al., 1998). However, organically complexed Sb(III) was found to represent 34% of total antimony in an acidic organic soil (Ettler et al., 2007). The neutral antimonite species, Sb(OH)_3 was found to readily bind to organic materials, with binding strengths higher than that of As(III) (Filella et al., 2002b; Buschmann and Sigg, 2004; Wilson et al., 2010). Several mechanisms have been proposed for Sb(III)

sorption to soil organic matter, primarily ligand exchange with phenolic groups (Tella and Pokrovski, 2009) and formation of a negatively charged complex with carboxylic acid functional groups (Buschmann and Sigg, 2004). Oxidation of Sb(III) by humic acids has been shown to be dependent on Sb(III)/DOC ratios and was consistent with a two binding site model including a strong binding site at low concentration inducing fast oxidation, and a weak binding site at high concentration inducing slower oxidation. Photoirradiation of natural samples spiked with Sb(III) suggest that the oxidation may be photodependent (Buschmann et al., 2005).

Antimonate was not initially predicted to have strong interactions with organic matter due to its net negative charge and results for Sb(V) retention by soil organic matter tend to be conflicting. Pilarski et al. (1995) reported no Sb(V) retention by humic acid at Sb concentrations below 10 mM. Conversely, significant capacity for Sb(V) retention was reported, with retention reaching <50% of total Sb(V), and higher Sb(V) levels being found in organic soils layers compared to deeper mineral layers (Steely et al., 2007; Tighe et al., 2005b). The mechanism of Sb(V) sorption by natural organic matter remains unresolved. However, work by Nakamuru et al. (2006) suggests that ligand exchange may be less significant than previously expected.

Tella and Pokrovski (2011) reported stable between Sb(V) and oxalic, citric, salicylic acids, catechol, xylitol and mannitol in aqueous solution. The authors suggested that the formation of stable complexes between Sb(V) and organic ligands requires the establishment of bidendate structures, in which Sb is bound via oxygen atoms from two adjacent functional groups, forming 5- or 6-membered chelate species. No complexing was detected with acetic, adipic and malonic acids and with

glycine in the pH range of natural waters suggesting that nitrogen containing ligands are not strong complexers of Sb (V).

1.3.6 Mobility of Sb in Soils

The relatively lower sorption strength of Sb(V) to organic and inorganic soil phases compared to Sb(III) accounts for the greater mobility of Sb(V), with more than 99% of the mobile Sb fraction in the Sb(V) oxidation state (Johnson et al., 2005; Lintschinger et al., 1998). Sb(V) was also shown to be the most stable form of antimony in aqueous solution over a wide range of pHs and pEs (Mitsunobu et al., 2006).

The sorption of Sb(III) to iron oxides is often accompanied by an oxidation to Sb(V) (Belzile et al., 2001; Leuz et al., 2006b). At low pHs this is not likely to influence mobility, as Sb(V) binding by iron oxides is favorable under acidic conditions. However, at higher pHs change in the oxidation state could result in a greater mobile antimony fraction as Sb(V) binding has been shown to be more pH dependent than sorption of Sb(III) (Leuz et al., 2006b).

Overall, dissolved forms of Sb represent less than 1% of overall soil Sb, particularly at low pHs (Muller et al., 2007). Readily exchangeable forms of Sb, represented by ammonium nitrate extractable Sb, also represented less than 1% of overall soil Sb (He, 2007; Hammel et al., 2000). This suggests a low mobility of Sb in soils.

The largest fraction of soil Sb, representing between 50 and 92% of total Sb, is associated with non crystalline oxides of iron, as indicated by oxalate extractable Sb (Johnson et al., 2005; Lintschinger et al., 1998; Tighe et al., 2007). Specifically

adsorbed Sb, measured by phosphate extraction represented only approximately 10% of total soil Sb (Ettler et al., 2007).

Ionic strength of the soil solution has a noticeable impact on Sb mobility. Increasing ionic strength tends to increase the number of non specific binding sites in soils, which was shown to significantly affect As(V) mobility (Smith, 1999). This non specific adsorption is highly dependent on the point of zero charge of the minerals present in the soil (Bowden et al., 1980). However, this trend was not observed for Sb(V). Instead, increasing ionic strength was shown to lower Sb(V) sorption by goethite. Additionally Sb(V) sorption by goethite was not consistent with outer sphere, non specific complexes and Leuz et al., (2006) suggest the sorption of Sb(V) inner sphere. Sb(III) mobility was not shown to be affected by ionic strength over a wide pH range, which is consistent with the high percentage of mobile Sb present in the antimonite form (Thanabalasingam and Pickering 1990; Wilson et al., 2010)

The presence of competing anionic species may also influence Sb mobility. Phosphate additions were shown to release substantial amounts of Sb(V) from soils where ligand exchange capacity was high (Kilgour et al., 2008; Nakamaru and Sekine, 2008). Nakamaur et al. (2006) reported a maximum Sb desorption of 40% upon phosphate addition and examinations of other soils show phosphate exchangeable Sb only represented 0.2-1.3% of total soil Sb (Nakamaru and Sekine, 2008). This suggests that ligand exchange mechanisms may not account for the total specifically adsorbed antimony in soil. If the conclusions, reported by Tighe et al (2005b), of 56% of total Sb being bound by organic materials holds then ligand exchange is unlikely to be the only specific adsorption mechanism operating for Sb(V) in organic soils. Thus

further examination is warranted to determine factors affecting binding and mobility of Sb in organic fractions to further assess bioavailability and potential exposure risks.

1.4. Bioavailability of Sb to Plants

1.4.1 Plant tissue concentrations

A large variation has been observed in bioavailable Sb fractions, as indicated by EDTA extractable Sb (He 2007). Tschan et al. (2009) suggest that the scatter found in Sb uptake can be attributed to these variations in bioavailable fractions. Even in heavily contaminated areas, plant tissue concentrations vary highly. For instance, in heavily contaminated soils containing <2000 mg kg⁻¹ total Sb, plant uptake was found to vary greatly from <5mg kg⁻¹ to 1367 mg kg⁻¹ depending on the species of plant (Baroni et al., 2000; Murciego et al., 2007).

Dominguez et al. (2008) reported antimony concentrations of woody plant leaves between 0.03 and 0.07 mg kg⁻¹ on soil that contained between 4.5 and 37.7 mg kg⁻¹ Sb, which corresponds to bioaccumulation coefficients of less than 0.03. Similar bioaccumulation rates were also obtained by Leduc and Gardou (1992) who analyzed plants growing on soil with the maximum Sb concentration of 105 mg kg⁻¹.

Hammel et al. (2000) observed antimony contents in plants and mobile Sb fractions 100 times higher in artificially polluted soils than in plants from the mining area. Similar high values are described by Vangronsveld et al. (1994) for vegetables, harvested in gardens in the vicinity of an antimony and lead recycling plant.

1.4.2 Uptake Mechanisms

In a review of available data for total soil Sb and plant tissue Sb, Tschan et al. (2009) found that plant Sb accumulation increases with the Sb concentration of soil

over a very wide range of concentrations; a relationship which can be approximated by a linear log-log regression model. However, a significantly better correlation was found between plant Sb concentration and soluble Sb instead of total soil Sb concentrations. Proportionality was also observed between extractable Sb concentration and plant tissue Sb (Hammel et al., 2000).

Unlike with arsenic, addition of phosphorous was found not to affect Sb uptake in maize (Tschan et al., 2008). This suggests that antimony uptake is not mediated by phosphorous specific membrane transporters. Sb uptake from hydroponic solution did not show a saturation point, which would have been predicted by ion specific transporters (Tschan et al., 2008).

Structural differences between antimonite and antimonate may account for differential uptake. Antimonite being neutral could potentially be transported into cells or root xylem by aquaporins (Filella et al., 2007). However, antimonate is negatively charged and thus cannot be transported by this mechanism. A standard membrane has an electrochemical gradient between -100 to -200mV. To overcome this potential difference the antimonate concentration outside the cell to be 2-3 orders of magnitude (Reid and Hayes 2003). Thus, without a specific ion transporter, symplastic uptake into plant roots is unlikely. Apoplastic transport however, would exclude most contaminants from transport into aerial parts of the the plants. The significant Sb concentrations found in shoot suggest that translocation from the root to the shoot is possible.

Plants may take up contaminants into their above ground parts not only through the roots, but also through aerial deposition of particulates onto the aerial plant parts.

A significant portion of the contamination then can become so tightly bound or incorporated that they cannot be removed even by vigorous washing (Hinton et al., 1995). This could potentially account for Sb concentrations in aerial portions of plants adjacent to road sides. A control experiment in which plants were grown under open-air conditions in pots with uncontaminated soil revealed that almost all this Sb uptake could be attributed to dust deposition onto the plant leaves. Robinson et al., obtained similar results at a highly polluted shooting range in Switzerland. The mechanism by which Sb could be absorbed systemically by plants from surface deposition has not been examined.

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CHAPTER 2

CHARACTERIZATION OF IRON – ORGANIC INTERACTIONS IN ORGANIC SOILS WITH INCREASING IRON TREATMENT

2.1 Introduction

Antimony (Sb) (atomic number 51) is a member of Group VA in the periodic classification of the elements. This element group also includes arsenic and phosphorus.

Traditionally, Antimony (Sb) exists as a trace element in the environment as a result of weathering from several antimony containing minerals. Sb is a chalcophilic element, meaning that it tends to concentrate in sulfide minerals and ores. In the lithosphere Sb exists as antimonosulphides, metal antimonides, and antimonoxides, principally stibnite (Sb_2S_3) and the transformation product valentinite. However, several other antimony containing minerals are found, such as senarmonite (Sb_2O_3) and stibiconite ($\text{Sb}_3\text{O}_6(\text{OH})$). In addition, Sb can be found associated in ores of Cu, Ag, Au, Hg, As and Pb (Alloway, 1995).

However the incidence of Sb in the environment has been increasing as the result of industrial uses. The history of anthropogenic input of Sb into the environment is comparable to the one of Pb because most Pb minerals are also rich in Sb (Shotyk et al., 1996). A peat core from an ombrotrophic Swiss bog revealed significant enrichments of Sb extending back to Roman times, indicating that anthropogenic fluxes of this metal have exceeded natural ones for more than 2000 years (Shotyk et al., 1996). Nowadays, Sb is used in its elemental form for the hardening of metals

(Fe, Ag, Au, Sn, Zn and Pb), specially combined with lead to make alloys (1-20% Sb) for the construction of batteries, bullets, cable sheathing, mechanical printing presses, machine bearing, etc. In addition, it is used in semiconductor technology for making infrared detectors and diodes. Antimony oxides, sulfides, sodium antimonate, and antimony trichloride are used in manufacturing flame-proofing compounds, manufacture of matches, paints, ceramic enamels, glass, and pottery. Organo-antimony preparations are used in humans against some tropical parasitic diseases. Other commercial uses for antimony include vulcanizers in tires and hardeners in plastics. Several studies have shown high concentrations of Sb in brake dusts (Iijima et al 2008; Gomez et al 2005)

The metalloid Sb exists in the oxidation states -III, 0, III and V, although in the environment it is most commonly found as Sb(III) and Sb(V). Both Sb(III) and Sb(V) hydrolyze easily in aqueous solution (Filella et al., 2002b). As a result, Sb(OH)_3 and Sb(OH)_6^- will be the most important species found in natural aqueous environments corresponding to the oxidation states Sb(III) and Sb(V), respectively. In water, Sb(III) is stable under reducing conditions and Sb(V) is stable under oxidizing to mildly reducing conditions. Since Sb may be present both as an anionic and as a neutral species, it will interact with the soil differently compared to heavy metal cations. The neutral species Sb(OH)_3 , is less soluble than Sb(OH)_6^- , and it may interact more favorably with negatively charged mineral surfaces and negatively charged organic matter ligands. Studies by Pilarski et al. (1995) and Buschmann and Sigg (2004) have confirmed that Sb(III) binds to humic acids. Sb(V) in solution is less likely to interact with soil organic matter (SOM) because both would be negatively charged at typical

soil pH. Several studies indicate that SOM would not play an important role in Sb binding (Filella et al., 2003; Crecelius et al., 1975). Pilarski et al. (1995) found no adsorption on humics by Sb(V) introduced as KSb(OH)_6 at concentrations $< 10 \text{ } \mu\text{mol L}^{-1}$. On the other hand, Gate and Richardson (1961) found that antimononic acid forms complexes with α -hydroxy acids and Lintschinger et al. (1998) found substantial amounts of Sb in soil associated with organic substances (alkaline and EDTA extraction at pH 7) and Tighe et al. (2005) showed a higher than expected sorption of Sb(V) by humic acid.

As with metal cations, anions sorb onto soil minerals that possess hydroxyl groups forming inner-sphere bonds. In this case, the reaction occurs through a ligand exchange process, which is favored at low pH values (Mukhopadhyay and Sanyal 2004). This occurs because the OH ligand at the mineral surface becomes a H_2O group, being easier to displace. Therefore, from the point of view of kinetics as well as equilibrium thermodynamics, low pH will favor anion adsorption and thus lower the mobility of anionic species (McBride, 1994).

Several authors have suggested that oxyanionic species could bind the negatively charged organic matter via a ternary complexation mechanism, in which cationic metals mediated the strong association between organic matter functional groups and oxyanions (Redman et al 2002; Thanabalasingam and Pickering 1986). This mechanism was demonstrated for phosphate interacting with organically complexed Fe and Mn (Leytem, and Westermann, 2005)

Chemical similarities between Sb and As may provide insight into the retention mechanism of Sb(V) by NOM. Mikutta and Kretzschmar (2011) used synthetic humic

colloids containing iron to examine the formation of ternary complexes between NOM, iron and arsenic. These authors found that 25-70% of total As was associated with iron in these synthetic complexes and used EXAFS to provide some of the first spectroscopic data of ternary complex structure. These synthetic organic complexes formed with ferric iron can be used to assess metal bridging of other oxyanions.

The structure of these ternary complexes is still lacking. Specifically, how the structure of the Fe-organic complex changes as Fe content changes. Gustafsson et al (2007) used EXAFS to show three significant iron signals in organic soils, an inner-core Fe–O/N interaction at about 2.02 Å, an Fe–C interaction in the second scattering shell at 3.00 Å, and a mean Fe–Fe distance at 3.37Å. Soils without iron addition showed features typical for iron (hydr)oxides; however, after treatment with iron(III) the EXAFS spectrum was dominated by organically complexed iron. The presence of an Fe–Fe distance, indicating close proximity of Fe atoms, in all samples showed that the major part of the organically complexed iron was hydrolyzed, most likely in a mixture of complexes. Analysis and modeling of the EXAFS spectra by Rose et al (1998) revealed that iron in organic colloids is poorly polymerized due to the complexation of Fe by the organic matter, since monodentate and bidentate complexes were detected. Hydrolyzed iron clusters in natural organic matter could provide a means for anion adsorption (Kizewski et al., 2010) and their formation and properties warrant further analysis. If these clusters influence antimony sorption, then K-edge XANES spectra would show similar energy shifts upon addition of Sb for both organic-Fe complexes and Fe-oxides when Sb interacts with these sites.

In this chapter, a XANES analysis with linear combination was used to identify

structural differences in iron added at several levels to an organic soil, differences which may be relevant to Sb sorption in organic soils. Considering that a XANES analysis of Fe speciation of organic colloids by Vilg -Ritter et al (1999) showed that organically complexed Fe is dominated by Fe (III) interactions, model Fe(III) organic complexes and Fe(III) oxide materials were characterized using a k-edge XANES analysis to determine the structure of Fe-organic interactions pre and post antimony addition. Fe(III) complexes of humic substances were examined to provide additional structural information on the formation of ternary complexes

2.2 Materials and Methods

2.2.1 XANES

All solid samples and references were ground and sieved to <100 m. Samples were diluted to 1% Fe by mass using sugar and coated on tape to form a thin layer. Thin layers were then stacked and sealed in kapton tape for analysis at the synchrotron. Liquid samples, primarily Fe-catechol, were placed in XRF containers and sealed with Mylar film.

A XANES analysis was conducted at the Cornell CHESS facility's F3 beam using a double-crystal Si (111) as a standard monochromator with a sagittal focusing second crystal. Fe K-edge XANES spectra were acquired in the energy range between 7085 and 7255 eV. The dwell time was 1 s, and the energy resolution was 0.5 eV. The spectra were acquired in fluorescence mode.

The spectra were background-corrected and edge-normalized using the software Athena (Ravel & Newville, 2005). The energy ranges used for background correction were 7085–7100 eV and those used for normalization of the spectra were

7210– 7250 eV (Fe). Edge energies were recorded from normalized spectra.

2.2.2 Examination of Fe complex formation in organic soils

2.2.2.1 Soil Treatments

Organic rich peat soils were collected from a farm in New Hampton, New York. All soils were sampled at random along an uncultivated 50m transect. Samples were taken between 0 – 18 inches below the soil surface, returned to the lab, sieved to < 2mm and dried before treatment with iron. pH measurements for each soil were taken in 1M CaCl₂. The Walkley-Black method (Allison 1965) was used to estimate organic carbon as a percent of whole soil. Free Fe-oxide levels in untreated soils were measured by the Coffin method using citrate-dithionate extractable Fe (Coffin, 1961) and cation exchange capacity was determined in an unbuffered barium chloride extract (Gillman, 1979). Soil physical and chemical properties are reported in Table 2.1.

Table 2.1 Chemical Characteristics of collected soils before iron-treatment. Parenthesis represents standard deviation of triplicate samples.

| pH | Organic Carbon (%) | CEC (meq kg ⁻¹) | Fe Oxides (mg kg ⁻¹) |
|------------|--------------------|-----------------------------|----------------------------------|
| 5.48 (.26) | 37.4 (3.4) | 594 (73) | 4429 (657) |

Iron treatment of soils was performed in bulk batches; 20kg of sieved, dried soils were weighed out into acid washed, 25L polyethylene containers. 20L of a 0.01M potassium nitrate (KNO₃) solution was added to create a 1:1 (m/v) soil suspension. The suspension was stirred vigorously for 5 minutes and then allowed to rest for 30 minutes before addition of iron. Ferric iron was added to the suspension, as iron chloride (FeCl₃·6H₂O), to reach 0, 0.01, 0.05 and 0.1% Fe, by mass. Suspensions were stirred vigorously for 30 minutes. Treated soils were allowed to air dry and

equilibrate at 25°C for four weeks.

2.2.2.2 Reference compounds

In order to distinguish iron oxide from organic iron complexes, common iron oxide minerals were synthesized. Goethite and 2 line ferrihydrite were synthesized according to methods described in Schwertmann and Cornell (1991).

The structure of the iron-humic complex was examined by comparison with model complexes of iron and organic ligands. Fe-catechol represented iron complexed by phenolic groups. Fe-catechol was synthesized by adding 1g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to a 1M solution of catechol. Commercially available iron- citrate was used as a model for iron bound by carboxylic acid functional groups.

2.2.2.3 Linear Combination analysis

Calculation of the contribution of different Fe species to overall Fe speciation in the Fe treated soils was carried out by linear combination fitting, using the routine included in Athena.

2.2.3 Characterization of Iron-Organic Colloids

2.2.3.1 Formation of Fe-humic acid complexes

Batch flocculation was performed on 6 L solutions containing 300mg L^{-1} humic acid (Sigma Aldrich) with 0.01M potassium nitrate (KNO_3) as background electrolytes. Solutions were titrated to pH 5.5 using 0.01M hydrochloric acid (HCl) and 0.01M sodium hydroxide (NaOH). Iron was added to the humic acid solutions in the form of iron chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in ratios of 1:10, 1:5 and 1:3 (Fe:humic acid m/m). After iron addition, samples were stirred slowly for 30 minutes to allow for flocculation to occur. Solutions were then centrifuged at 6,000 rpm for 45 minutes

and filtered to collect all solid materials. The recovered flocculants were freeze dried and stored at room temperature in sealed polyethylene bottles before XANES analysis.

2.2.3.2 Mossbauer Spectroscopy

In order to provide additional structural information, iron Mossbauer spectroscopy was performed on the colloidal organic-Fe complexes at Villanova University's Department of Geology. An untreated organic soil was used as a reference. Room temperature measurements were obtained on all four samples.

2.2.4 Determination of Sb interactions with organically complexed Fe

An additional XANES analysis was performed to examine the Fe spectra of the soil and colloid samples pre- and post- antimony addition. Additional sensitivity to 0.4 eV was achieved with the addition of a channel cut crystal on the F3 beamline. A longer integration time of 5 seconds was also used to reduce noise from the increased sensitivity.

2.2.4.1 Sb Treatments

Soils samples and Fe-organic colloid samples were treated with 800 mg kg⁻¹ Sb. Soil samples were treated by placing 1 kg of soil in a sealed 2 L pot. Sb(V) was added as solid potassium antimonate (KSb(OH)₆) sufficient to bring the soil Sb concentration to 800 mg kg⁻¹. Control pots received no Sb treatment. Pots were allowed to equilibrate for 2 weeks in greenhouse conditions with regular watering.

Fe colloids were spiked to 800 mg kg⁻¹ Sb by adding 50mL of a 80 mg L⁻¹ solution of potassium antimonate (KSb(OH)₆) to 5g of each sample. Samples were shaken for 24 hours, then centrifuged, decanted and allowed to air dry for one week.

2.3 Results and Discussion

2.3.1 Structure of Fe complexes in organic soil

Table 2.2 – Linear combination factors of reference spectra expressed as a percentage of the soil spectra

| Soil Fe Treatment | Linear Combination Factor (%) | | |
|----------------------|-------------------------------|-------------|------------|
| | Ferrihydrite | Fe-Catechol | Fe-Citrate |
| 0.0% | 51.0 | 28.4 | 20.6 |
| 0.01% | 44.6 | 30.7 | 24.7 |
| 0.05% | 41.2 | 31.9 | 26.9 |
| 0.1% | 32.4 | 35.8 | 31.8 |

Figure 2.1 shows the XANES spectra for the iron treated soils. Reference compounds found to be significant contributors by the linear combination analysis are included on each spectrum. Linear combination factors (LCF) were used to represent the contributions of the reference spectra to the soil Fe spectra at each of the soil Fe levels (Table 2.2). The linear combination analysis shows a clear increase in the contribution of the Fe-catechol and Fe-citrate spectra as soil Fe concentration increased. This is accompanied by a decrease in the contribution of the ferrihydrite spectra. Crystalline Fe species were not significant in the Fe treated peat soils according to the linear combination analysis. This indicates an increase in organic complexation as Fe addition to the peat soil increases. Comparison of XANES spectra for soils and iron oxides is shown in Figure 2.3. Considering the declining LCF of the Fe oxides, there is no evidence of formation of additional Fe oxide formation with increasing Fe treatment.

Fe pre-edge peaks of the soil and Fe-oxide spectra are shown in Figure 2.4. The pre edge peaks represent 1s to 3p transitions and are highly dependent on

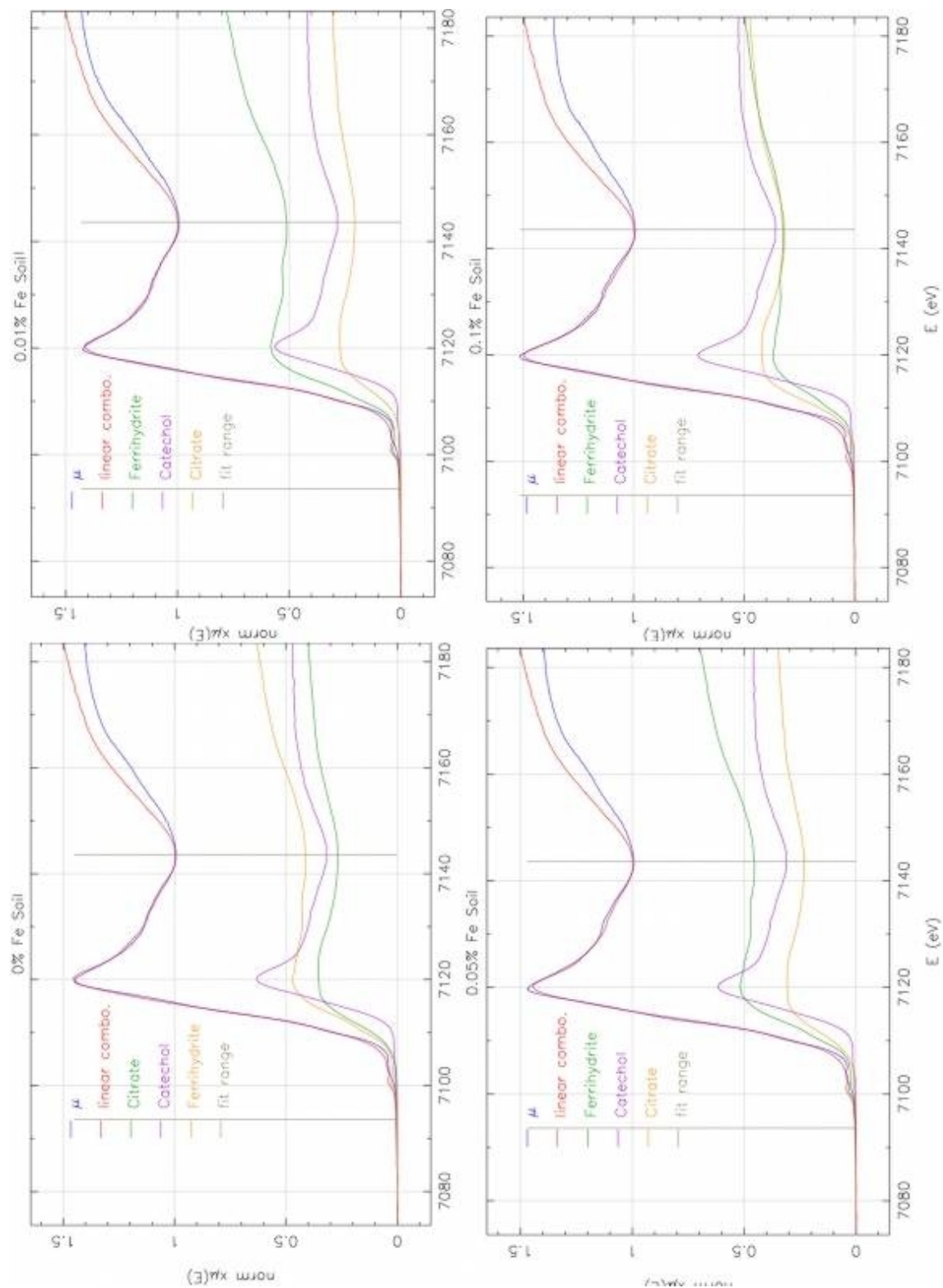


Figure 2.1- XANES Linear combination spectra of Fe treated soils with organic Fe references and oxides of Fe

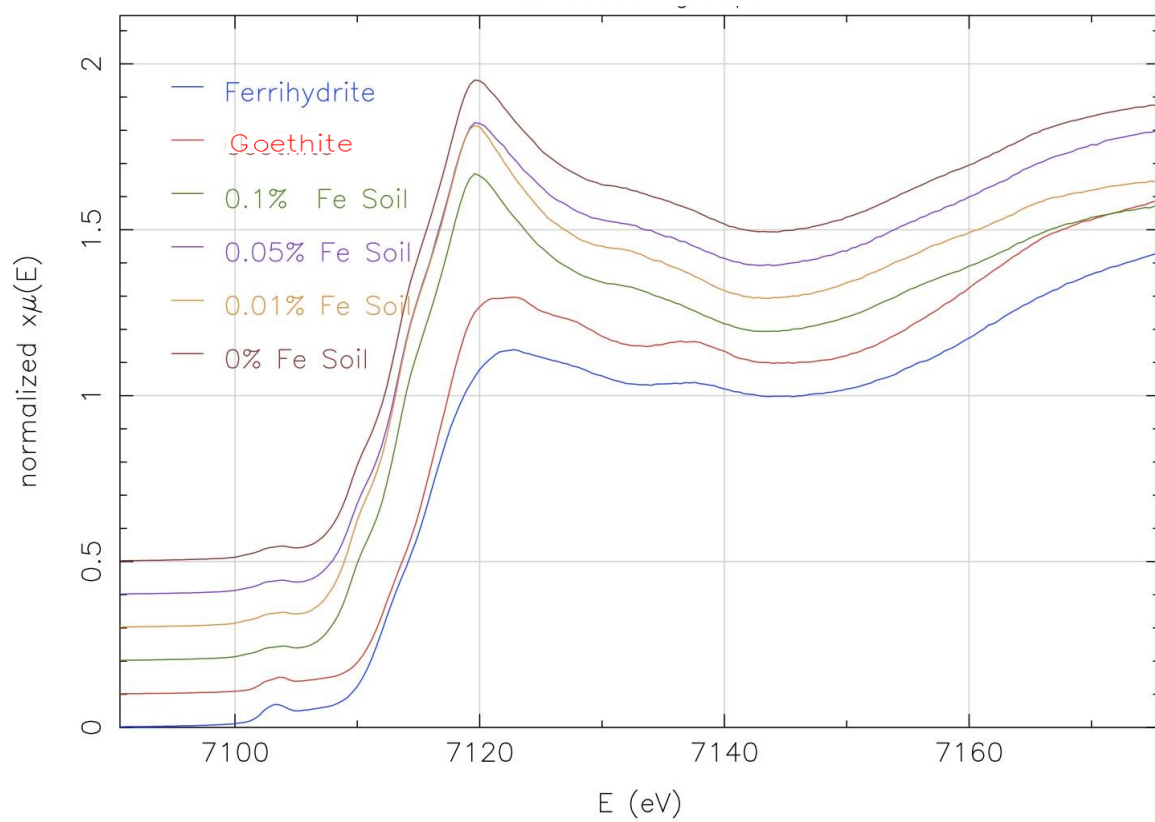


Figure 2.2 – Stacked XANES Spectra of iron treated soils and Fe oxides reference compounds

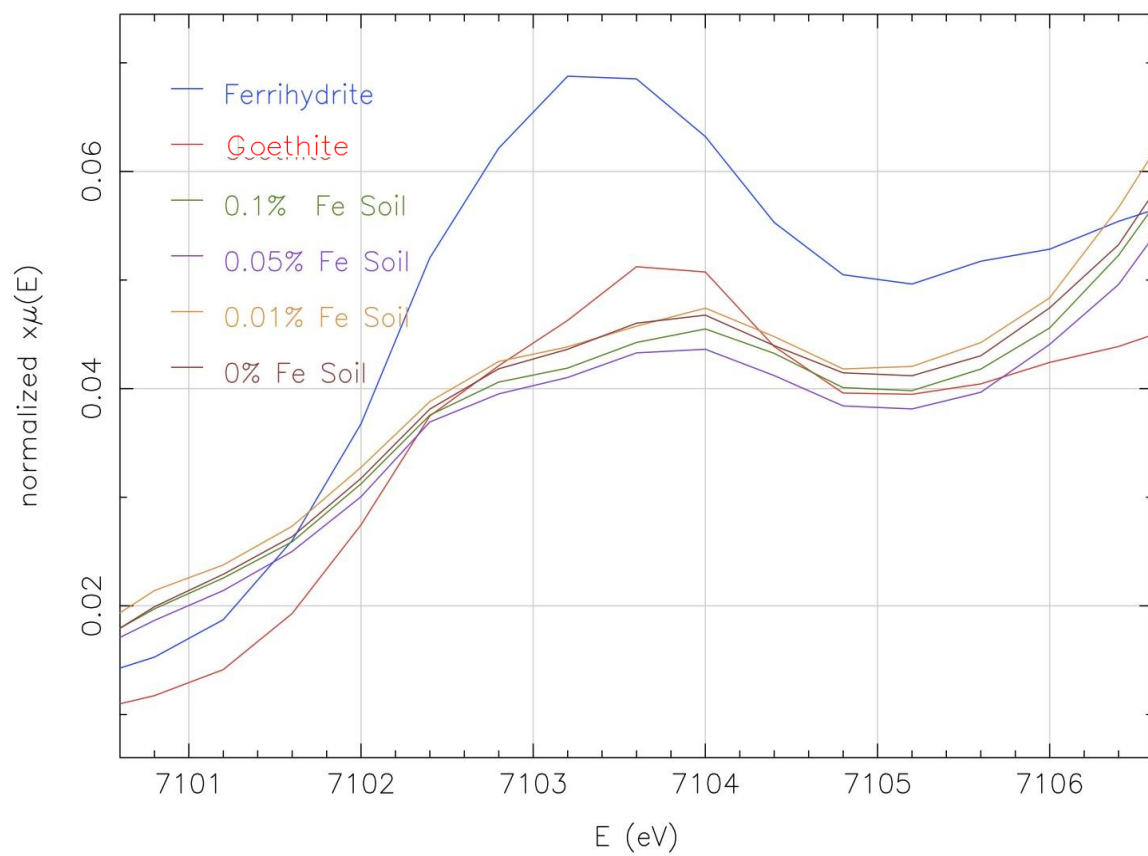


Figure 2.3 - XANES Pre-edge peaks of Fe treated soils and Fe oxide reference material

coordination chemistry. Small, less intense bands are indicative of octahedral coordination of Fe, which are centro-symmetric with no p-d mixing allowed and only quadrupolar transitions (Berthet et al., 1988). In contrast, large well-defined peaks would indicate tetrahedral coordination due to lack of central symmetry. Ferrihydrite reference spectra showed a larger peak in the pre edge region than goethite. These peaks are not large enough to indicate tetrahedral coordination but the change in height could potentially indicate slight distortion of symmetry found in goethite, which would result from p-d mixing and dipole transitions in the pre-edge (Manceau and Gates 1997). This decrease in coordination symmetry is expected due to ferrihydrite's non-crystalline nature versus goethite's highly crystalline one. Soil samples showed a more goethite-like pre-peak region, which structurally this suggests that Fe contained in organic soils maintains undistorted octahedral coordination.

Edge energies (E_0) for reference compounds and soil samples are reported in Table 2.3. Edge energy for Fe-catechol was higher than E_0 for Fe-citrate indicating that the Fe-bond lengths are longer when complexed by phenolic functional groups compared to carboxylic acid groups. The slight shift in the energy between Fe-catechol and Fe-citrate indicates that iron bound by catechol, and by extension polyphenols, is in a much more confined state, resulting in a sharper peak.

Table 2.3 - Edge energy for reference and soil samples obtained from XANES Spectra.

| References | E_0 (Ev) | Soils | E_0 (Ev) |
|--------------|------------|-----------------------|------------|
| Ferrihydrite | 7115.6 | 0.0% Fe Treated Soil | 7113.6 |
| Goethite | 7116.4 | 0.01% Fe Treated Soil | 7113.6 |
| Fe-Citrate | 7115.0 | 0.05% Fe Treated Soil | 7113.2 |
| Fe-Catechol | 7118.0 | 0.1% Fe Treated Soil | 7113.6 |

2.3.2 Characterization of Iron-Organic Colloids

Spectra for the humic-Fe colloids are shown in Figure 2.4. All colloidal species, regardless of content, showed the same somewhat flattened spectra as observed for the spectrum of Fe-citrate. Additionally, these spectra lack the sharp peaks at the K- edge that would signify the presence of phenolically complexed Fe. This suggests that Fe-colloidal species are composed primarily of Fe complexed with carboxylic acid moieties.

Mössbauer spectra with spectral fits are shown in Figure 2.5. Spectral parameters are tabulated in Table 2.4. The Organic-Fe colloid samples showed spectra consistent with a 2 site model. The three humic-Fe colloids exhibited similar spectra consistent with the superposition of two quadrupole doublets (Table 2.4). Both doublets correspond to high spin Fe^{3+} in different coordination environment. The site with the smaller quadrupole splitting ($\text{QS} \sim 0.5 \text{ mm/s}$) corresponds to monomeric iron in high coordination symmetry environment, while the site with $\text{QS} \sim 1.00 \text{ m/s}$ is consistent with distorted coordination symmetry similar to that observed in iron dimers. Figure 2.5d shows the Mossbauer spectra of the organic soil sample. Sites 1 and 2 for Fe in this organic soil are similar to those observed for the humic-Fe colloid samples. In addition the soil sample gives signatures for a small amount of high spin Fe^{2+} ($\text{IS} \sim 1.11 \text{ mm/s}$ and $\text{QS} \sim 2.70 \text{ mm/s}$). The XANES results in this experiment suggest that Fe in the colloidal samples is organically complexed, particularly by carboxylic acid functional groups. Ferrihydrite and goethite do not appear to contribute significantly to these materials. However, the results of the Mossbauer spectroscopy suggest that Fe in the colloidal samples is forming dimers or trimers as

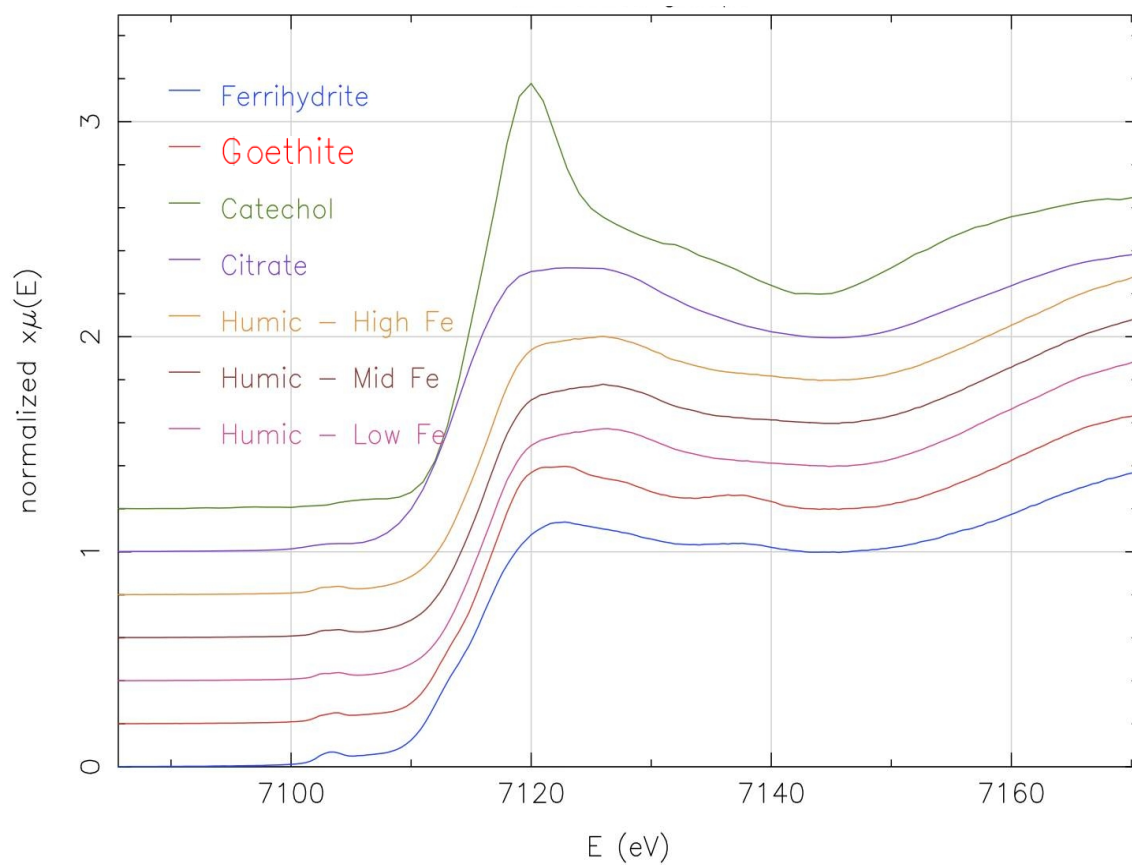


Figure 2.4 – Stacked XANES Spectra of Fe – Humic Acid colloidal materials with Fe reference materials

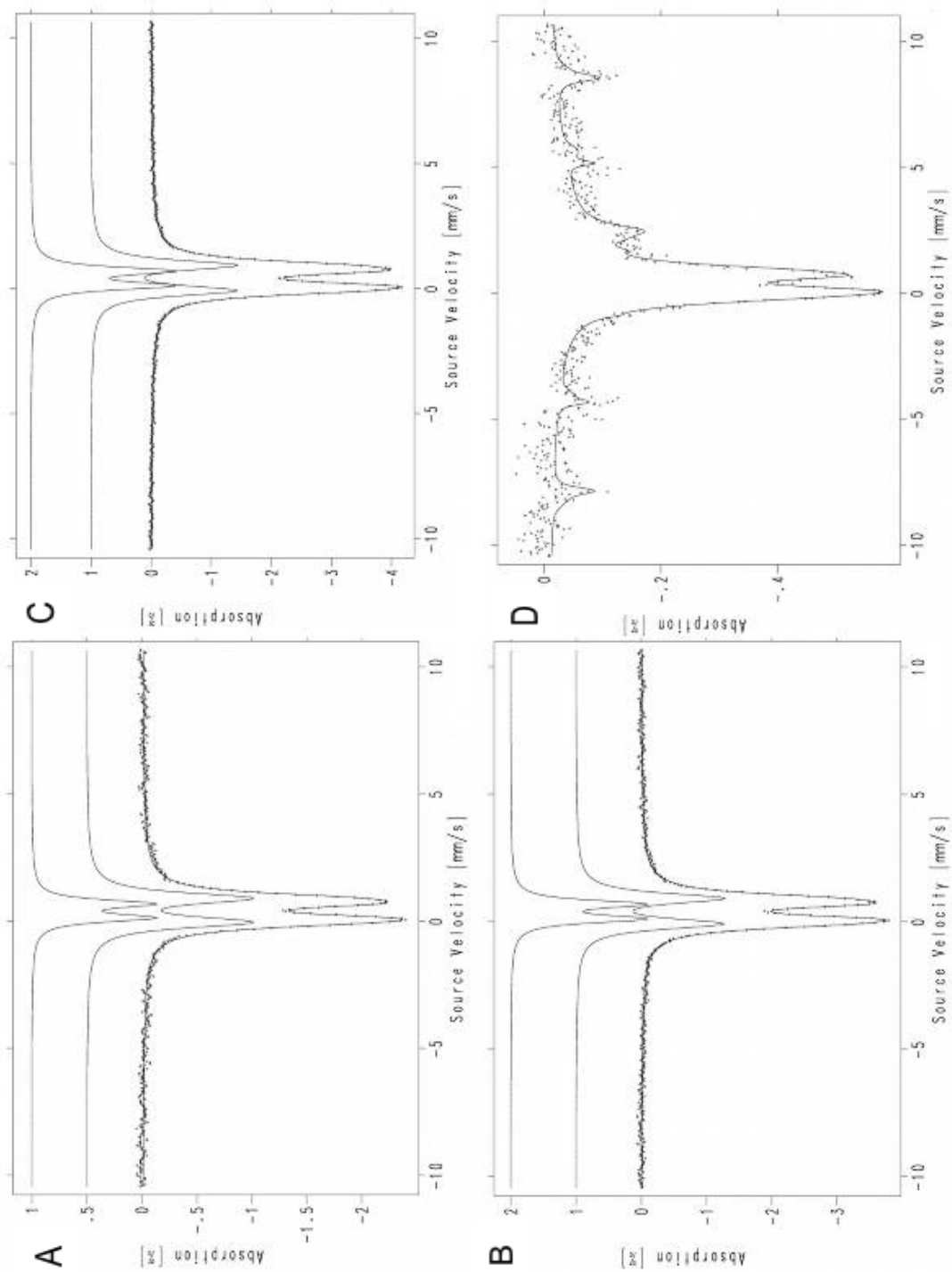


Figure 2.5 - Mossbauer Spectra of (A) Low Fe Organic Colloids (B) Mid Fe organic colloids (C) High Fe organic colloids (D) untreated organic soil

Table 2.4 - Summary of spectral parameters from Mossbauer spectroscopy of Fe-humic colloids and organic soil

| Low Fe-Humic Colloid | | Site | Γ (mm/s) | δ (mm/s) | ΔE_Q or e (mm/s) | Fractional Area | |
|-----------------------|------|-------------------------|---------------------------------------|--------------------|--------------------------------------|-----------------------------|--------------------|
| | | 1 | 0.526 | 0.419 | 0.946 | 0.66 | |
| | | 2 | 0.370 | 0.397 | 0.559 | 0.34 | |
| Mid Fe-Humic Colloid | | Site | Γ (mm/s) | δ (mm/s) | ΔE_Q or ϵ (mm/s) | Fractional Area | |
| | | 1 | 0.492 | 0.416 | 1.017 | 0.57 | |
| | | 2 | 0.377 | 0.400 | 0.592 | 0.43 | |
| High Fe-Humic Colloid | | Site | Γ (mm/s) | δ (mm/s) | ΔE_Q or e (mm/s) | Fractional Area | |
| | | 1 | 0.504 | 0.414 | 1.002 | 0.60 | |
| | | 2 | 0.36 | 0.398 | 0.583 | 0.40 | |
| Organic Soil | Site | H_{eff} kOe | Dist. of H_{eff} (kOe) | Γ (mm/s) | δ (mm/s) | ΔE_Q or e (mm/s) | Fractional Area |
| | 1 | 0 | 0 | 0.6 | 0.42 | 0.95 | 0.41 |
| | 2 | 0 | 0 | 0.44 | 0.40 | 0.56 | 0.27 |
| | 3 | 0 | 0 | 0.6 | 1.11 | 2.70 | 0.16 |
| | 4 | 506 | 10.4 | 0.2 | 0.38 | -.063 | 0.08 |
| | 5 | 518 | 40.1 | 0.3 | 0.54 | -.509 | 0.08 |

well as a certain proportion of iron monomers. XANES results suggest that Fe clusters are occurring in the colloidal samples but are not large enough to register as ferrihydrite or goethite. Similar iron species are observed in the soil sample but do not represent all the iron in the soil (Figure 2.5D). Thus, the colloidal materials potentially represent the organically complexed fraction of soil Fe.

2.3.3 XANES analysis of Sb treated samples.

The XANES spectra for the Fe oxide reference samples and oxides treated

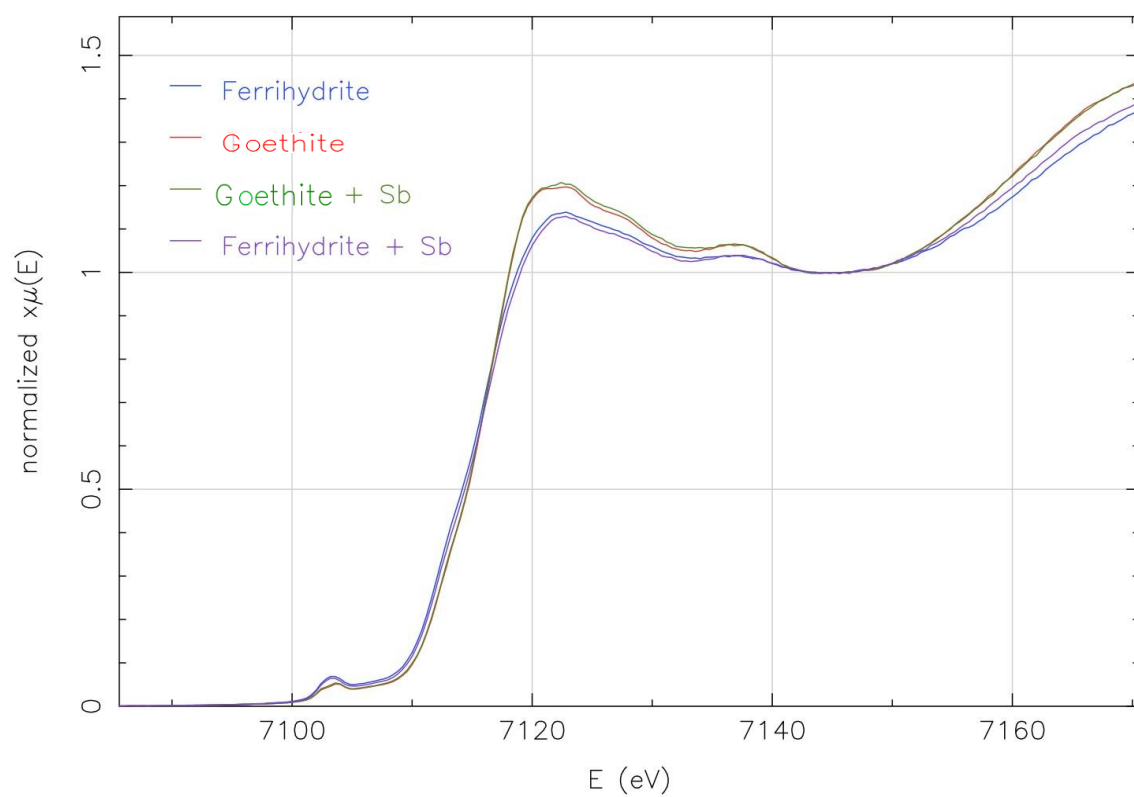


Figure 2.6 - XANES spectra of Fe oxide references pre and post Sb treatment

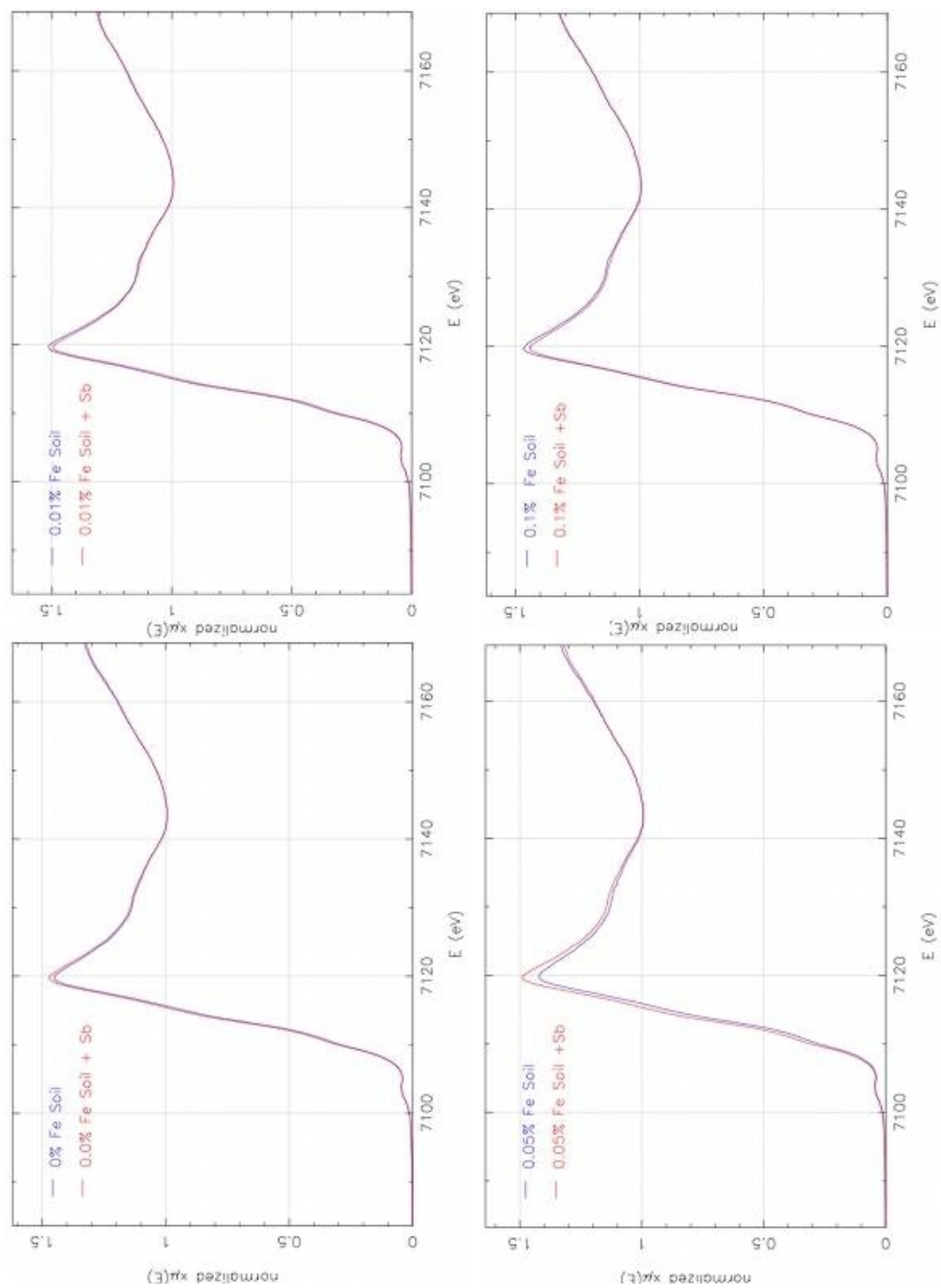


Figure 2.7 – XANES spectra of Fe treated soils pre- and post-Sb treatment

with Sb are shown in Figure 2.6. No observable changes in XANES spectra occurred upon the addition of Sb. Soil spectra before and after Sb treatment are shown in Figure 2.7. Changes in edge energies were not observed between untreated soils and soils containing Sb (Table 2.5). This is likely due to the XANES analysis being dominated by either Fe-organic or Fe-Fe interactions; therefore Fe-Sb interactions in this system are obscured by the more dominant interactions so that they are not detectable by Fe XANES analysis.

Table 2.5 - Edge Energies of soils and Fe oxides pre and post Sb treatment

| | E_0 pre Sb treatment (Ev) | E_0 post Sb treatment (Ev) |
|-----------------------|-----------------------------|------------------------------|
| Goethite | 7115.6 | 7116.4 |
| Ferrihydrite | 7116.4 | 7116.8 |
| 0% Fe Treated Soil | 7113.6 | 7113.6 |
| 0.01% Fe Treated Soil | 7113.6 | 7113.6 |
| 0.05% Fe Treated Soil | 7113.2 | 7113.6 |
| 0.1% Fe Treated Soil | 7113.6 | 7113.6 |

2.4 Conclusions

Iron amendments made to highly organic soils resulted in an increase in organically complexed Fe. Both phenolic and carboxylic Fe complexes increased, but no evidence of additional Fe oxide formation was detected in the treated soils. Iron complexed by phenol groups showed a more constrained electron environment which could be significant regarding the availability of these iron groups to form metal bridging complexes. Iron bound by carboxylic acid functional groups is not constrained in this way. It also appears that Fe bound by organic matter is in a highly symmetrical coordination sphere based on the low pre edge peaks in the XANES.

The Mossbauer spectra presented in Chapter 2 suggest the formation of small

clusters of Fe, particularly trimers, in humic materials. These clusters do not register as goethite or ferrihydrite according to the XANES analysis presented here. NOM has been shown to limit the Fe hydrolysis to the oligomeric stage. Vile-Ritter et al. (1999) found that Fe oligomers consisted primarily of single-corner-sharing trimers, stabilized by bidentate bonding with carboxylic acid functional groups. The Mossbauer spectra also show a significant portion of Fe bound as monomers with high coordination symmetry. Overall this suggests two distinct iron sites in natural organic materials. A proposed structure for each of these two sites is presented in Figure 2.8.

Scheinost et al. (2006) used EXAFS spectroscopy to demonstrate the chemical interactions between Fe oxides and Sb. The authors found Fe atoms at radial distances of 3.10 and 3.56 Å from Sb atoms; which, according to the authors, are in line with edge-sharing and bidentate corner-sharing linkages between Sb(V) and iron oxide octahedra. Complimentary to these observations, Ackerman et al. (2009) showed that Sb sorbs to oxides via a combination of edge and corner sharing adsorption complexes on the Fe oxide surface. However, the EXAFS results of Sb(V) sorbed to goethite reveal only the edge-sharing sorption complex (Scheinost et al 2006). Fe clusters shown in Figure 2.8 have a greater number of available corner and edge sites when compared to the monomeric iron complexes shown.

Corner and edge sharing of Fe octahedral has been shown to be important in the formation of ternary complexes of arsenic oxyanions and natural organic matter and thus could be relevant in the strength of Sb interactions with these Fe complexes in natural organic materials (Mikutta and Kretzschmar, 2011).

No changes were observed in the Fe XANES upon the addition of Sb. This is

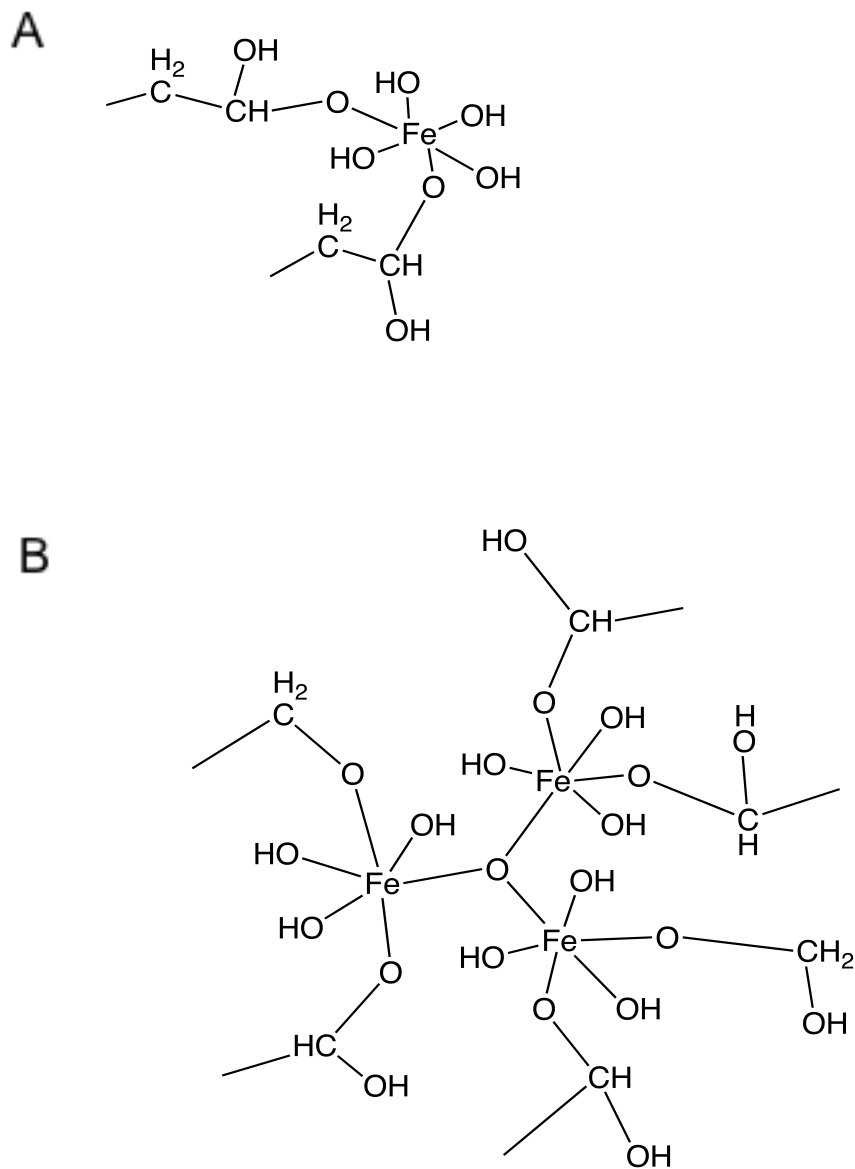


Figure 2.8 - Proposed structures of Fe complexes in soil organic matter. (A) Iron monomer bidentately bound to carboxylic acid functional groups (B) Corner sharing of Fe to form trimers. Each Iron octahedral is bidentately bound to a carboxylic acid group. Adapted in part from data presented in Mikutta and Kretzschmar (2011) and Vile-Ritter et al. (1999).

likely due to the fact that XANES examines the average bonding environment of the analyzed element in complex matrices. Thus, the interactions of Fe with the organic functional groups as well as other iron atoms dominate the spectra. It is difficult to determine inter-atomic distances in a XANES analysis. If Sb metal bridging complexes are being formed, then an EXAFS analysis to examine bond distance in the oxygen sphere surrounding the iron atoms could produce clearer results about the structure of Fe in organic materials upon the addition of Sb.

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CHAPTER 3

THE EFFECT OF PHOSPHATE ON ANTIMONY INTERACTIONS WITH IRON

(III) -HUMIC COMPLEXES

3.1 Introduction

Antimony (Sb) is a metalloid in group 15 of the periodic table. Antimony has been used with increasing frequency in the production of consumer products, such as metal alloys, plastics, and flame-retardants for electronics (Filella et al., 2002a; Cheng et al., 2010). As a result, antimony is increasingly released into the environment through industrial pollution as well as post-consumer processes. The increasing concentrations of Sb in the environment, coupled with its potential toxicity makes understanding the environmental reactions of Sb essential for protecting human and environmental health.

Antimony shares several chemical similarities with arsenic and phosphorous. Most notably, Sb is found in the environment in either the +3 or +5 oxidation states. Sb(V) is the most common oxidation state in oxic environments and occurs as an oxyanionic species, antimonate ($\text{Sb}(\text{OH})_6^-$). As a result of its net negative charge, antimonate was not initially predicted to have strong interactions with natural organic matter (NOM). Thus, results for Sb(V) retention by soil organic matter tend to be conflicting. Pilarski et al. (1995) reported no Sb(V) retention by humic acid at Sb concentrations below 10 mM. Conversely, A growing body of evidence shows significant interaction between Sb(V) and NOM with retention reaching <50% of total Sb(V), and higher Sb(V) levels being found in organic soils layers compared to deeper

mineral layers (Steely et al., 2007; Tighe et al., 2005b). The mechanism of Sb(V) sorption by natural organic matter remains unresolved. However, a study in Japan, which was measuring sorption coefficients for antimony under various conditions, showed a lower than expected decrease in sorption upon the addition of phosphate anions, which could not be explained by anion competition or displacement because the addition of nitrate had no effect on antimony mobility. The phosphate exchangeable Sb accounted for 20-40% of soil-sorbed Sb. However, the authors suggest that the observed desorption percentages of Sb should be the upper limit of the Sb desorption by phosphate addition, but more than 50% of added Sb was strongly fixed to the soil solid phase and was not readily exchangeable with phosphate. This led the authors to the conclusion that Sb sorption mechanisms should not be explained only by a ligand exchange like phosphate adsorption (Nakamaru et al., 2006). Spuller, et al. (2007) also observed the effect of phosphorus on antimony speciation.

Several authors have suggested that oxyanionic species could bind the negatively charged organic matter via a ternary complexation mechanism, in which cationic metals mediated the strong association between organic matter functional groups and oxyanions (Redman et al 2002; Thanabalasingam and Pickering 1986). This mechanism was demonstrated for phosphate interacting with organically complexed Fe and Mn (Leytem, and Westermann, 2005). It is possible that the differences between observed and expected phosphate exchangeable Sb is related to the formation of these ternary complexes.

Mikutta and Kretzschmar (2011) used ferric iron complexes of humic acid (HA) to assess the formation of ternary complexes. This work uses similar iron-humic

complexes to examine the competition between antimony and phosphorous for sorption sites in organic materials.

3.2 Materials and Methods

3.2.1 Formation of Humic-Fe colloids

Batch flocculation was performed on 6 L solutions containing 300mg L⁻¹ humic acid (Sigma Aldrich) with 0.01M potassium nitrate (KNO₃) as background electrolytes. Solutions were titrated to pH 5.5 using 0.01M hydrochloric acid (HCl) and 0.01M sodium hydroxide (NaOH). Iron was added to the humic acid solutions in the form of iron chloride (FeCl₃·6H₂O) in ratios of 1:10, 1:5 and 1:3 (Fe:Humic Acid m/m). After iron addition, samples were stirred slowly for 30 minutes to allow for flocculation to occur. Solutions were then centrifuged at 6,000 rpm for 45 minutes and filtered to collect all solid materials. The recovered flocculants were freeze dried and stored at room temperature in sealed polyethylene bottles.

3.2.2 Adsorption

Antimony solutions were prepared with potassium antimonate (KSb(OH)₆) at Sb concentrations between 5 and 15mg L⁻¹. Both sets of solutions contained .01M KNO₃ as a background electrolyte. Solutions were titrated to pH 5 using 6M HCl. All solutions were prepared in glassware analytically cleaned with phosphate free detergents and acid washed.

75mg of materials were weighed out into centrifuge tubes. 7.5mL of antimony solution was added to the tubes and were placed in an end over end shaker for 24 hours. Tubes were removed and centrifuged at 6,000rpm for 20 min. Supernates were collected for analysis.

Freundlich isotherms were constructed for each of the materials and Freundlich constants were determined graphically.

In order to distinguish iron oxide sorption behavior from sorption behavior of the iron-humic complex, sorption isotherms were obtained for Goethite. 75mg of goethite was shaken with 7.5mL of the Sb solutions containing 5-15mg/L. Tubes were again shaken for 24 hours, centrifuged and supernates collected for analysis

Adsorption behavior of an organic soil, previously described by McBride 2001, was examined to place adsorption of synthesized organic colloids into field context.

Sb concentrations of collected solutions were determined using inductively coupled plasma spectroscopy at 206.83nm. Standards were prepared using potassium antimonate ($\text{KSb}(\text{OH})_6$) in 0.01M KNO_3 .

3.2.3 Adsorption with the addition of phosphate

The influence of phosphorous on antimony sorption behavior was examined by performing sorption experiments with solutions that contained both antimony and phosphorous in varying ratios.

Sb sorptions were run at antimony to phosphate ratios of 1:1 and 1:2. Solutions were prepared by dissolving potassium antimonite ($\text{KSb}(\text{OH})_6$) and calcium phosphate in 0.1M KNO_3 and titrating to pH 5. Antimony in the solutions contained between 5mg L^{-1} and 15mg L^{-1} Sb at both ratios. Phosphate concentrations ranged from 5 to 15mg L^{-1} PO_4 in 1:1 solutions and from 10 to 30mg L^{-1} Sb in 1:2 solutions.

Sorption procedures were identical for competitive sorption experiments as for control sorptions. 75mg of the synthesized materials, goethite, or the organic soil were

shaken with 7.5mL of solution for 24 hours followed by centrifugation for 20 minutes and collection of supernates for analysis by ICP.

3.3 Results

3.3.1 Adsorption

Figure 3.1 shows the sorption isotherms obtained for the sorptions of antimony. Freundlich constants are shown in Table 3.1. The organic soil had a significantly lower K value than all the colloidal samples and goethite ($P < 0.001$). There was no significant difference in K values between the 1:10 and 1:5 Fe:HA colloids. The colloidal sample with the highest iron treatment (1:3) showed the greatest sorption capacity with K values significantly higher than all samples, including the goethite and oil samples ($P < 0.001$). Goethite had a significantly greater K value than the organic soil, and the 1:10 and 1:5 Fe:HA colloids ($P < 0.001$).

n values from the Freundlich isotherms are also shown in Table 3.1. n values for the three colloidal samples were not significantly different.

3.3.2 Effect of Phosphate

Values of the Freundlich constants for the competitive sorption study are reported in Table 3.2

Table 3.1 - Freundlich constants for adsorption of Sb

| Constant | Goethite | Soil | 1:10 Fe:HA complex | 1:5 Fe:HA complex | 1:3 Fe:HA complex |
|----------|------------------|------------------|-----------------------|----------------------|----------------------|
| K | 1.34 (0.123) | 0.031 (.009) | 0.1 (.002) | 0.111 (0.005) | 2.44 (0.454) |
| n | 0.185 (0.050) | 0.753 (0.549) | 0.903 (0.125) | 1.054 (0.183) | 1.126 (0.201) |

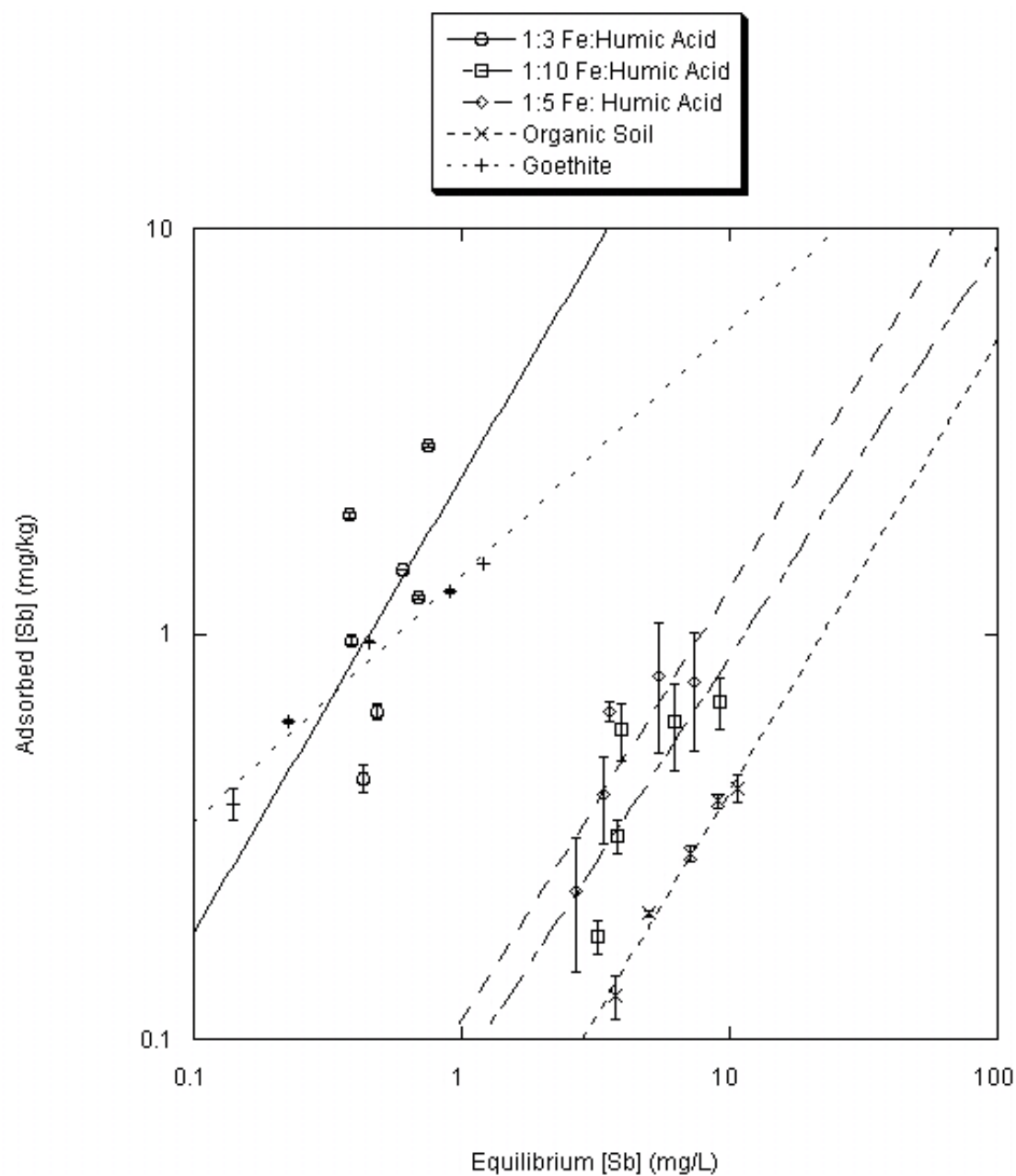


Figure 3.1 – Freundlich isotherms for adsorption of Sb on Fe-HA complexes and reference materials.

Table 3.2 – Freundlich Constants for adsorption of Sb with phosphate addition

| Sample | No Phosphate | | 1:1 Sb:P | | 1:2 Sb:P | |
|-------------------------|--------------|------|----------|------|----------|------|
| | K | n | K | n | K | n |
| Goethite | 1.34 | 0.19 | 0.47 | 0.49 | 0.71 | 0.42 |
| Soil | 0.03 | 0.75 | 0.05 | 1.27 | 0.04 | 1.11 |
| Low Fe Complex | 0.1 | 0.9 | 0.05 | 0.86 | 0.06 | 0.64 |
| Intermediate Fe Complex | 0.3 | 1.05 | 0.11 | 0.23 | 0.18 | 0.06 |
| High Fe Complex | 2.44 | 1.13 | 0.26 | 0.35 | 0.21 | 0.04 |

Figure 3.2 shows the competitive sorption isotherms for goethite. K values for Sb adsorption by goethite were negatively affected by the addition of phosphate at equal concentration to Sb. This negative affect on Sb adsorption is lessened when phosphate is present in excess as shown by the increase in K values between adsorptions performed at a 1:1 Sb:P ratio and the adsorptions performed at a 1:2 Sb:P ratio.

Figure 3.2 also shows the competitive sorption isotherms obtained for the organic soil. K values for adsorptions performed with phosphate present were not significantly different from those observed for adsorption without phosphate present. Competition isotherms of the colloidal samples are shown in Figure 3.3 and values for the Freundlich constants are reported in Table 3.2

Figure 3.3a shows the isotherms for the low Fe material. A significant difference in K values was observed between the control and both samples containing phosphate ($P=0.014$). Samples containing equal parts phosphate and antimonate were not significantly different from samples with phosphate in excess of antimonate, with respect to K values. The material with the intermediate Fe content (Figure 3.3b) showed an identical trend with the K value at both phosphate levels being significantly

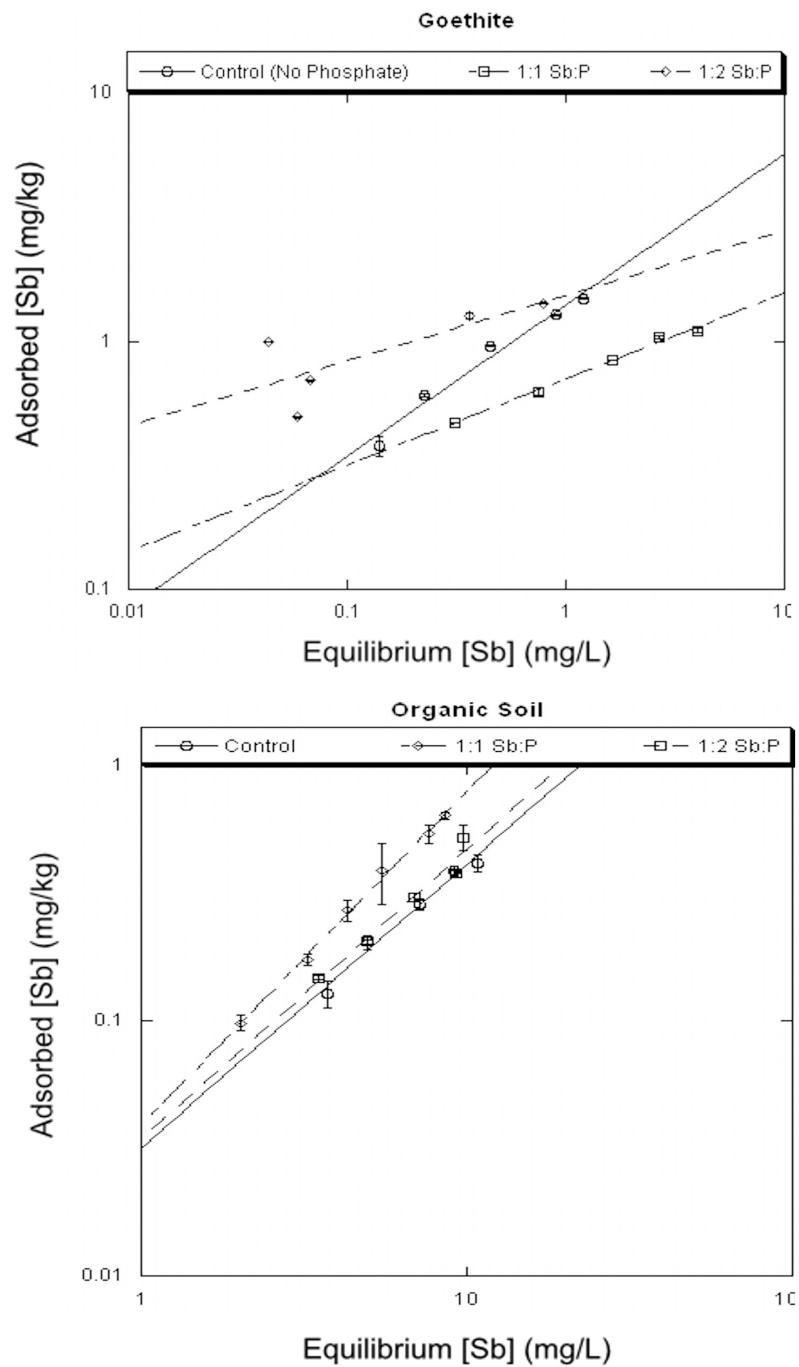


Figure 3.2 – Adsorption isotherms for Sb on Goethite, and an organic soil with phosphate addition

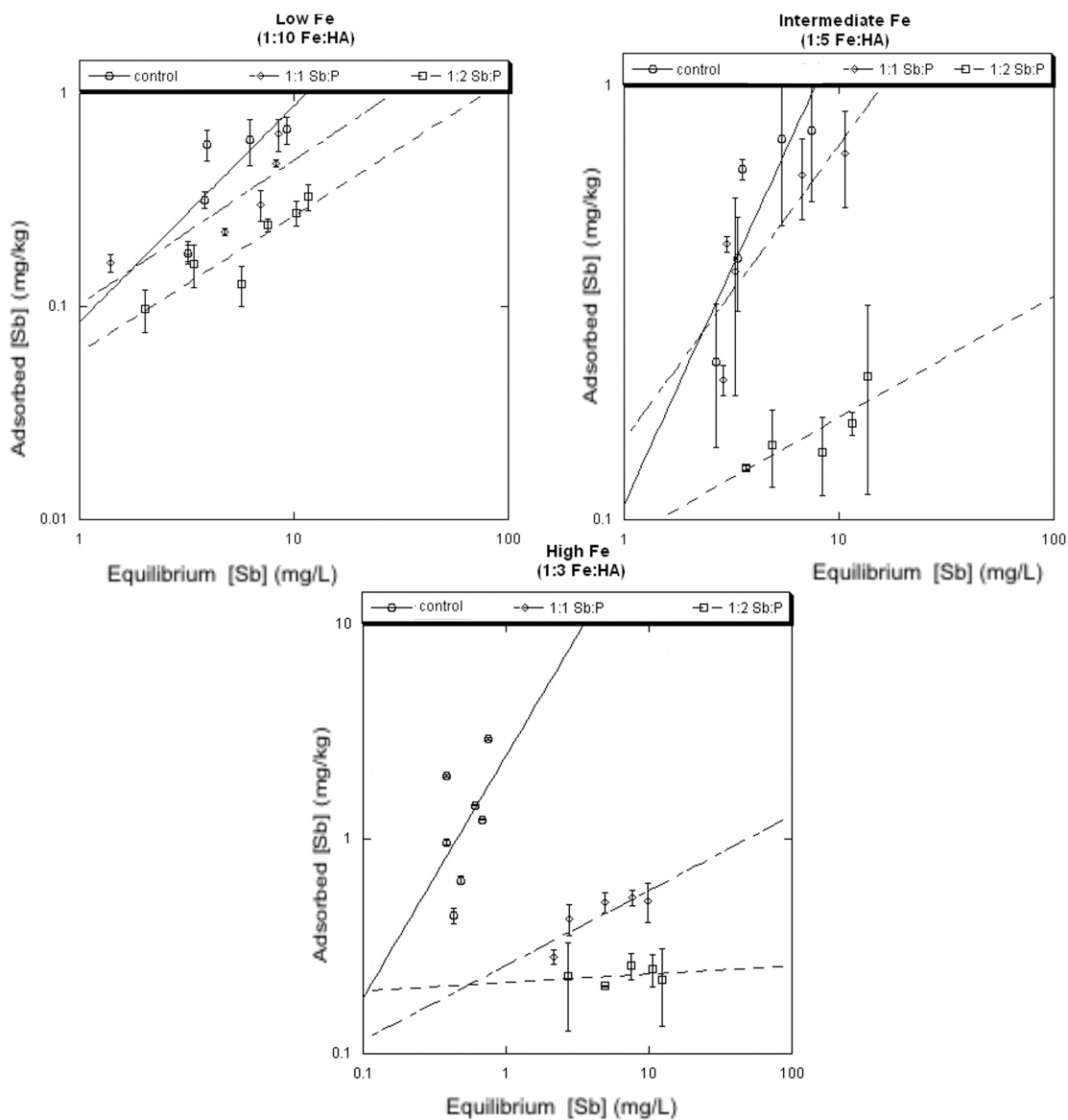


Figure 3.3 - Adsorption isotherms for Sb on Fe-H.A complexes with the addition of phosphate.

lower than controls ($P=0.021$), but with no significant difference observed between the different phosphate levels. The high Fe materials (Figure 3.3c) showed the greatest drop in Sb sorption when phosphate was introduced. Both the sorptions containing phosphate showed a significant decrease in K from sorption without phosphate ($P<0.001$, $P<0.001$). K values for these sorptions were not statistically significant from one another.

3.4 Discussion

K values in the Freundlich isotherm represent the sorption capacity of a given adsorbate for the adsorbent. This indicates that the soil sample had a significantly lower sorption capacity for Sb than the pure Fe-humic complexes and goethite. The colloidal species with the 1:10 and 1:5 Fe:HA treatments were not significantly different from one another in terms of sorption capacity but were significantly higher than the whole soil. A significant jump in sorption capacity was observed between the colloids the highest Fe and the other colloidal species. The sorption capacity of this material was also higher than for goethite.

Considering Goethite's well documented interactions with Sb (Gleyzes et al., 2002; Manaka, 2005), it was expected that goethite would have the greatest sorption capacity of all the materials tested. However, the colloidal species with the highest iron treatment showed the greatest sorption capacity.

Structural differences in the Fe-humic complexes may account for the trend in sorption capacity observed in this experiment. Two distinct iron sites were observed in the organic materials examined in chapter 2. Monomeric iron complexes contain fewer edge and corner sites capable of interacting with Sb when compared to

oligomeric Fe complexes that form in these materials. Changes in the proportions of these sites in the materials could greatly affect Sb retention if ternary complexes are forming. Specifically, an increase in the proportion of Fe clusters in these materials, as Fe content increases might account for the increase in sorption capacity in the high Fe materials. The decline in sorption of Sb by goethite upon addition of a phosphate suggests competition for binding sites on Fe oxides. The decline in sorption was greatest when Sb and the competing phosphate ion were in equal concentrations, Further addition of competing ions caused recovery of sorption capacity.

The drop in sorption capacity in the materials containing 1:10 and 1:5 Fe:HA when phosphate was present in either concentration suggest that phosphate is preferentially adsorbed by these materials and is not displaced by Sb.

In the humic materials containing the highest iron additions, addition of phosphate at both ratios caused a very significant decrease in Sb sorption capacity. This suggests that P outcompetes Sb for sorption sites at all the phosphate concentrations examined.

3.5 Conclusions

The results of this experiment shine light on the interactions of organically complexed Fe and antimony. In a whole organic soil only small amount of Sb were adsorbed suggesting a lack of binding sites. On the contrary, organic colloids containing significant amounts of Fe sorbed much larger concentrations of Sb. At higher Fe levels, the sorption capacity even rivals that of goethite, which has been shown to adsorb significant amount of Sb (Leuz et al.,2006b Scheinost et al., 2006). Overall, Fe content of organic materials could be playing a very significant role in the

soil chemistry of Sb, and these interactions may be dependent on the structure of the iron complex.

Competition between Sb and phosphate in these materials seems to be more complicated. In pure Fe oxide materials the presence of phosphate caused a decline in the strength of Sb interactions but overall sorption capacity was variable based on the relative concentrations of phosphorus and Sb. By contrast, in an organic soil containing little iron sorption capacity of Sb remained relatively constant with addition of phosphate as a competing oxyanion. In the pure Fe-HA complexes, phosphate caused significant reductions in sorption capacity and sorption strength at both phosphate concentrations examined. The ability of phosphate to significantly prevent Sb interactions with Fe-HA complexes implies that Sb-Fe-HA ternary complexes are weaker than ternary complexes with phosphate. This would further imply that the discrepancy between the predicted phosphate exchangeable Sb and those observed by Nakamaru et al. (2006) is not related to ternary complex formation, as Sb in these complexes appears to be greatly affected by phosphate. It seems that Sb ternary complexes are weak in relation to other mechanisms of Sb adsorption. The whole soil system remained largely unaffected by the presence of phosphate where the pure Fe-HA complexes showed the opposite. Therefore, direct interactions between Sb and organic ligands may be more significant than previously expected.

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CHAPTER 4

EXAMINATION OF IRON DEPENDENT ADSORPTION AND MOBILITY OF ANTIMONY IN ORGANIC SOILS

4.0 Introduction

Antimony (Sb) is a metalloid in group 15 of the periodic table. As such, Sb has many chemical similarities to arsenic and phosphorus. For instance, arsenic and Sb both occur in the environment in two primary oxidation states, +3 and +5, (Filella et al., 2002a). The chemical similarities of Sb and As also mean that Sb has toxicological mechanisms similar to arsenic, including carcinogenicity (Casiot et al., 2007; Gebel, 1997). Antimony has been used with increasing frequency in the production of consumer products, such as metal alloys, plastics, and flame retardants for electronics (Filella et al., 2002a; Cheng et al., 2010). As a result, antimony is increasingly released into the environment through industrial pollution as well as post-consumer processes. The increasing concentrations of Sb in the environment, coupled with its potential toxicity makes understanding the environmental reactions of Sb essential for protecting human and environmental health.

In oxic environments the primary oxidation state of Sb is +5, which occurs as an oxyanion (Sb(OH)_6^-). The net negative charge of natural organic matter would generally tend to repel anionic species. Yet, organic materials in soils have been shown to contribute to the retention of Sb (Tighe et al., 2005b). The mechanism by which these interactions occur is not fully understood. However, ligand exchange processes have been shown to be important in the retention of chemically similar anionic phosphate species. Nakamura et al. (2006) found that a smaller than expected

percentage of Sb(V) was phosphate-exchangeable in a whole soil system. The octahedral structure of the antimonate anion means that charge density is focused on the antimony atom itself, which indicates that ligand exchange mechanisms would not tend to be as strong for antimonate compared to the tetrahedral phosphate, where charge density is on the outer oxygen atoms. The presence of a fraction of adsorbed Sb that was not phosphate-extractable Sb indicates that ligand exchange processes are not the only interactions of antimony in soil.

The ability of humic acids to sorb anions such as phosphate has been shown to be related to the aluminum (Al) or iron (Fe) content of the humic materials (Larsen et al., 1959; Levesque and Schnitzer, 1967; Wada and Higashi, 1976; Owusu-Bennoah and Acquaye, 1989). The formation of metal bridging complexes could potentially provide a mechanism for Sb(V) retention in organic soils.

The role of colloids of Fe and natural organic matter (NOM) in anion adsorption is still being examined. Colloidal Fe and NOM appear to be important in the binding and transport of As species (Riggle and von Wandruszka, 2002). Colloidal Fe also plays a role in P cycling but it is unclear whether this is primarily due to a co-precipitation of phosphate with the Fe colloids or due to a sorption mechanism, or both. On the other hand, Sb binding shows strong correlation to colloids of NOM and it has been suggested that more than 50% of Sb in the environment exists in small organic complexes (Riggle and von Wandruszka 2002; Klitzke and Lang 2009). These colloids are unlikely to be pure phases but rather a complex of organic matter and Fe. This could indicate that the mobility and availability of Sb in the environment is subject to the stability of these colloids (Kretzschmar and Sticher 1997).

This work examines effect organically complexed Fe has on sorption of Sb(V) by organic soils. Extraction of Sb from soils was also used to assess the influence of organic Fe on mobility of Sb(V). If Fe in organic colloids influence Sb(V) sorption, then differences should be observed as iron content on the colloids is varied. Likewise, mobility of Sb(V) should be dependent on Fe concentration and the extractant type used to solubilize it.

4.2 Materials and Methods

4.2.1 Soil Sampling and Characteristics

Organic rich soils were collected from a farm in New Hampton, New York. All soils were sampled at random along an uncultivated 50m transect. Samples were taken between 0 – 18 inches below the soil surface. Soils were dried to constant mass and sieved to <2mm before treatment with iron. pH measurements for each soil were measured in 1M CaCl₂ (1:2m/v) using a glass pH electrode (Thermo 9142BN). The Walkley-Black method (Allison 1965) was used to estimate organic carbon as a percent of whole soil. Free Fe-oxide levels in untreated soils were measured by the Coffin method using citrate-dithionate extractable Fe (Coffin, 1961) and cation exchange capacity was determined in an unbuffered barium chloride extract (Gillman, 1979). Soil chemical properties are reported in Table 4.1.

Table 4.1 Chemical Characteristics of collected soils before iron-treatment. Parenthesis represents standard deviation of triplicate samples.

| pH | Organic Carbon (%) | CEC (meq kg ⁻¹) | Free Fe Oxides (mg kg ⁻¹) |
|------------|--------------------|-----------------------------|---------------------------------------|
| 5.48 (.26) | 37.4 (3.47) | 594 (73.7) | 4430 (657) |

4.2.2 Iron Treatment

For iron treatment of soils, 10g of sieved, dried soils was weighed out into analytically cleaned flasks. 1L of a .01M potassium nitrate (KNO_3) solution was added to create a 1:100 (m/v) soil suspension. The suspension was stirred vigorously for 5 minutes and then allowed to rest for 30 minutes before addition of iron. Ferric iron was added to the suspension, as iron chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), to reach 0, 0.005, 0.01, 0.05 and 0.1% Fe, by mass. Above this range, precipitation of iron hydroxides was evident and below 0.01% Fe, no visible aggregation of soluble or suspended organic materials was observed. Suspensions were stirred vigorously for 30 minutes before being transferred to tubes and being centrifuged for 30 minutes at 6000RPM. Supernatants were decanted and filtered. Treated soils were allowed to air dry and equilibrate at 25°C for two weeks. The concentration of iron in the supernatants was analyzed by flame atomic absorption spectroscopy using air-acetylene at a wavelength of 248.3nm. The concentration of Fe retained was calculated by difference in mass of Fe added to soil and the mass of Sb found in supernatants divided by the total mass of soil.

4.2.3 Antimony Sorption

Antimony solutions containing between 5 and 50ppm Sb(V) were made using potassium hexahydroantimonate ($\text{KSb}(\text{OH})_6$) with .01M KNO_3 as a background electrolyte. 50mL of the antimony solutions were added to 5g of the dried, treated soils. The pH of the resulting soil solution was measured with a glass electrode, and the suspensions were titrated to pH 5.5 using 0.1M HCl and 0.1M NaOH. Samples were shaken for 24 hours in order to reach equilibrium (Pilarski et al., 1995). Samples were then centrifuged at 3000rpm for 15 minutes. Supernatants were decanted and

filtered through Whatman 42 filters before Sb analysis. The soil was allowed to air dry at room temperature for 3 days prior to mobility analysis.

Final Sb concentrations in the solution were measured by flame atomic absorption spectroscopy at 206.8nm. Concentrations below detectable range for AA were re-examined using standard additions. Equilibrium adsorbed (solid) Sb was calculated by difference using the equilibrium solution Sb concentration.

A separately soil sample was treated and used for each of the four replicate isotherms. Equilibrium solution and solid Sb concentrations were used to construct Freundlich isotherms for each soil (Tighe, 2005b). Values of Freundlich constants for each replicate isotherm were determined graphically and mean Freundlich constants were calculated for each iron treatment level. A Kruskal-Wallis test with post-hoc Bonferroni adjustment was used to test for significant differences between Freundlich constants of all 5 soil Fe treatments.

4.2.4 Mobility Analysis

In order to determine the mobility of Sb in each of the treated soils, soils amended with Sb at various concentrations were randomly assigned to groups and extracted with additional solutions.

The mobile fraction of Sb was assessed by shaking 5g of soil from the original Sb sorption experiment with 50mL of 1M ammonium nitrate (NH_4NO_3) (Hammel et al., 2000).

5g of Sb treated soils were shaken with 50mL of 0.1M potassium phosphate (KH_2PO_4) to extract Sb bound by ligand exchange mechanisms (Nakamuru et al., 2006)

0.1M potassium pyrophosphate has been used to extract organically complexed Fe and was thus used to examine the release of Sb upon extraction of organic Fe. 5g of Sb treated soils were shaken in 50mL of 0.1M potassium pyrophosphate (Bascomb, and Thanigasalam, 1968).

Finally, in order to assess the role of iron mediated Sb adsorption, 5g of treated soils were extracted with 0.2M ammonium oxalate (Schwertmann, 1964). Oxalate has been used to extract free forms of Fe, including non-crystalline and crystalline phases of Fe oxides. Extraction with oxalate thus indicates interactions with total free Fe forms.

Concentrations of Sb extracted by these reagents were determined using flame atomic absorption spectroscopy and used to calculate the remaining adsorbed Sb concentrations.

All extractions were performed for 24 hours to allow equilibrium to be reached between adsorbed and extracted Sb. Equilibrium conditions were necessary for construction of distribution curves for each extraction. Constraining the intercept of the curve to 0 allowed the partition coefficient (K_d) to be calculated from the slope of the distribution curve for each extraction. Mean distribution coefficients were calculated for each of the extractions in all 5 soil Fe treatments.

4.3 Results

4.3.1 Fe treatment

Table 4.2 - Adsorbed Fe concentrations calculated. Parentheses represent standard deviation of 4 replicate samples

| Fe Treatment | 0.0% | 0.005% | 0.01% | 0.05% | 0.1% |
|--------------------------|--------------|----------------|----------------|-----------------|------------------|
| Adsorbed [Fe] (mg/kg) | 0.0 (0.0) | 47.2 (1.78) | 93.1 (3.81) | 455.7 (30.6) | 502.46 (52.1) |

Table 4.2 shows the mean concentration of Fe retained in the soil at each Fe treatment level obtained through a mass balance calculation. Fe concentrations in the soil increased linearly from 0 - .05% (m/m) added Fe before leveling off above the .05% Fe treatment. These results were fitted to a Langmuir function with maximum adsorption ($[\text{Fe}]_{\text{max}}$) occurring at 737 mg kg^{-1} (Figure 4.1).

4.3.2 Sb Sorption

Figure 4.2 shows the constructed Freundlich functions for each of the 5 treated soils. The mean Freundlich constant values for all Fe treatments are summarized in Table 4.3. According to the Kruskal-Wallis test, significant differences exist between median K values for the 5 soil Fe treatments ($P < 0.001$). Post-hoc Bonferroni adjustment shows no significant difference between the 0% and 0.005% Fe treatments ($P = .544$) or the 0.5% and the 0.1% Fe treatments ($P = .862$). All other differences were found to be significant ($P < .001$).

The only significant difference in n values occurred between the 0.01% and 0.05% Fe treatments ($P = .031$). All other differences in n values were found to be statistically insignificant according to the Kruskal-Wallace test.

Table 4.3 - Mean Freundlich constants. Parentheses represent standard deviation of 12 replicate samples

| Soil Fe Treatment | 0.0% | 0.005% | 0.01% | 0.05% | 0.1% |
|-------------------|----------------|----------------|----------------|----------------|----------------|
| K | 2.01 (1.18) | 2.25 (0.99) | 4.79 (0.86) | 7.54 (0.76) | 7.55 (1.19) |
| n | 0.82 (0.23) | 0.80 (0.13) | 0.77 (0.06) | 0.88 (0.04) | 0.86 (0.05) |

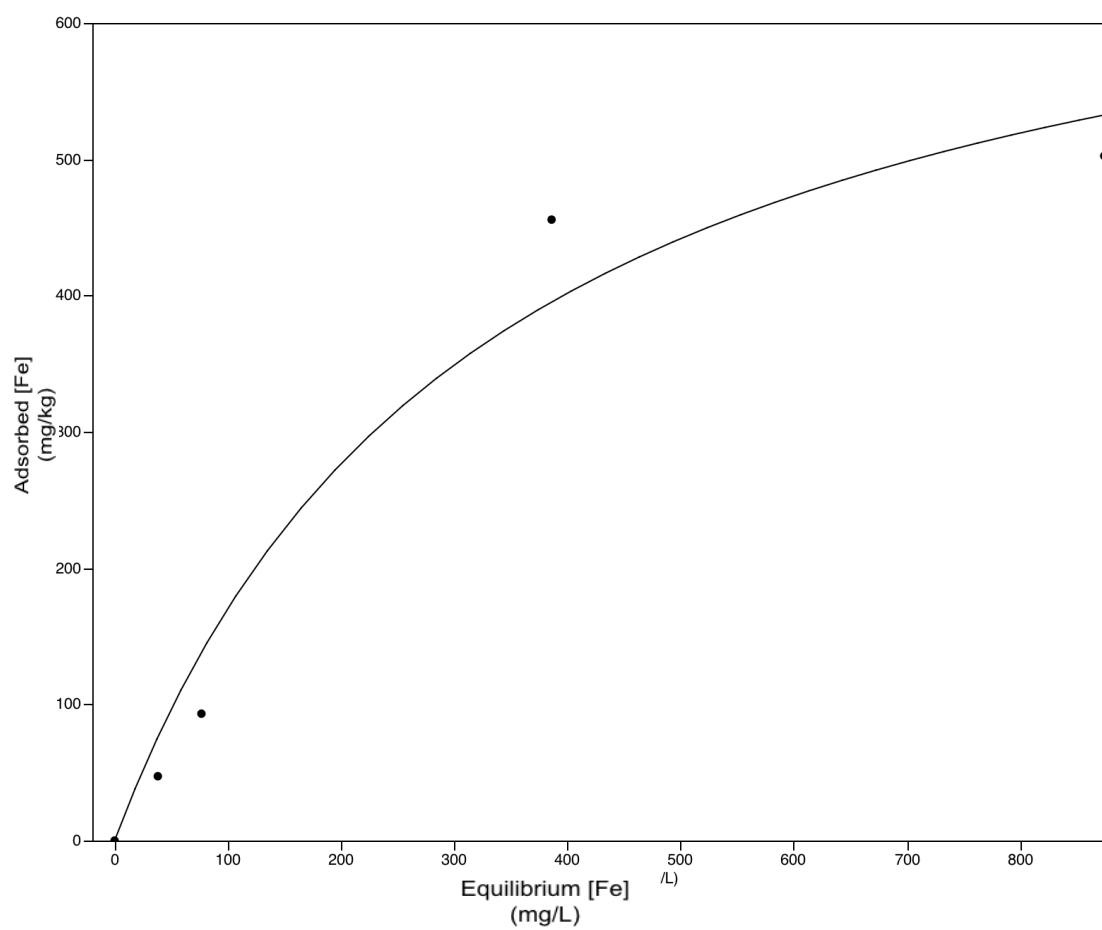


Figure 4.1 – Fe retention isotherm fitted with a Langmuir function ($R^2 = .9761$)

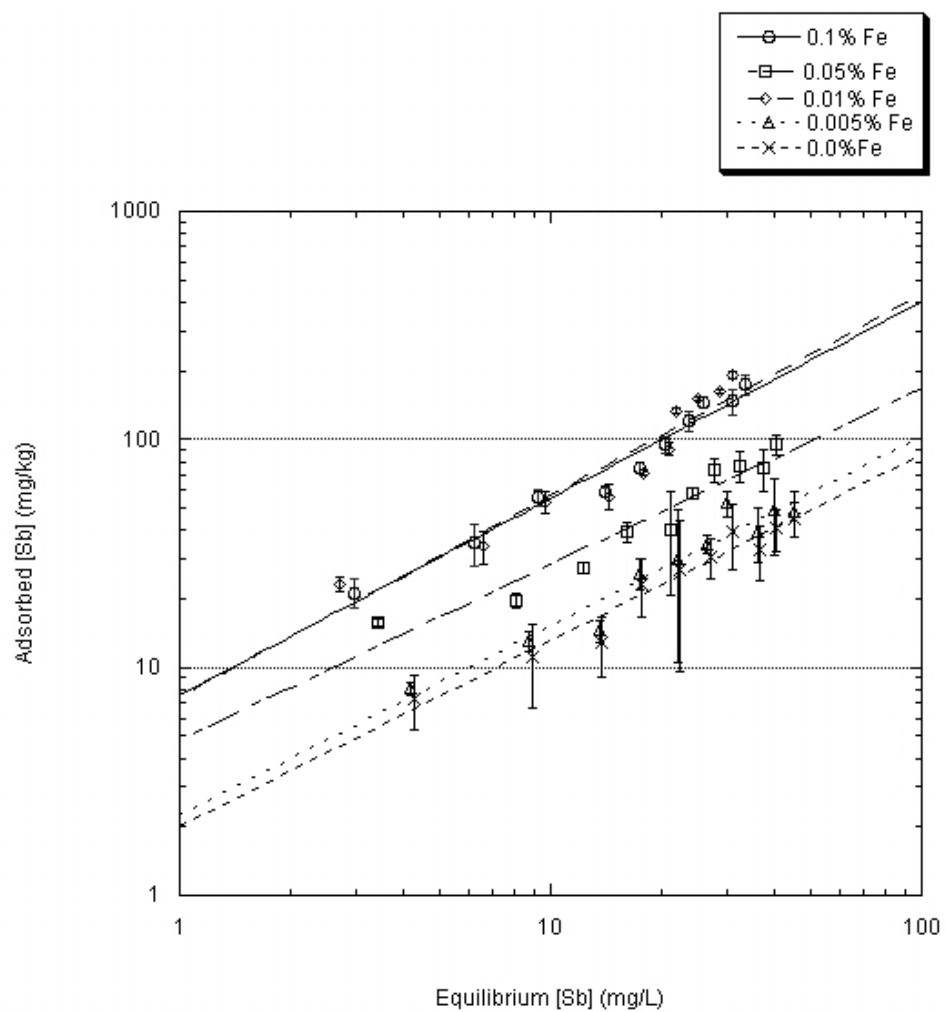


Figure 4.2 - Mean Sb retention Freundlich Isotherms for all soil Fe treatments.
 R^2 0% - .8780; 0.005% - .9333; 0.01% - .8696; 0.05% - .95013; 0.1% - .88016

Figure 4.3 shows the mean K values for Sb sorption on each Fe-treated soil plotted against the mean soil Fe concentration as determined using the difference between initial Sb and final Sb in solution (Table 4.2). These results are also estimated by a Langmuir function.

4.3.3 Sb mobility

The calculated partition coefficients (K_d) for each of the extraction treatments are shown in Table 4.4 K_d is defined as the ratio of adsorbed Sb to Sb in solution.

Table 4.4 -Sb Partition Coefficients (K_d) for extraction treatments of Fe treated soils. Parentheses represent standard deviation of triplicate samples

| Soil Fe Treatment | NH ₄ NO ₃ | Phosphate | Oxalate | Pyrophosphate |
|-------------------|---------------------------------|-----------------|----------------|----------------|
| 0.0% | 8.23 (1.67) | 1.05 (0.37) | 6.23 (2.11) | 8.18 (0.45) |
| 0.005% | 9.44 (1.58) | 1.14 (0.38) | 7.82 (1.59) | 5.37 (0.29) |
| 0.01% | 19.31 (3.93) | 2.44 (.50) | 3.49 (0.42) | 4.73 (0.82) |
| 0.05% | 29.88 (5.03) | 28.53 (0.87) | 2.71 (0.37) | 3.62 (0.45) |
| 0.1% | 32.35 (2.39) | 20.51 (0.19) | 2.51 (0.23) | 6.64 (1.28) |

4.3.3.1 Mobile Fraction

Figure 4.4 shows the partition curves for the 5 treated soils after extraction with NH₄NO₃. Partition coefficients increased only slightly between 0 and 0.005%Fe treatment. However, addition of Fe, at higher levels, in the range of aggregate formation increased partition coefficients noticeably from controls. Additionally, the partition coefficients increased between the 0.01%, 0.05% treatments. The increase in partition coefficients was not as significant between the 0.05% and the 0.1% Fe treatments, but still notable (Table 4.4).

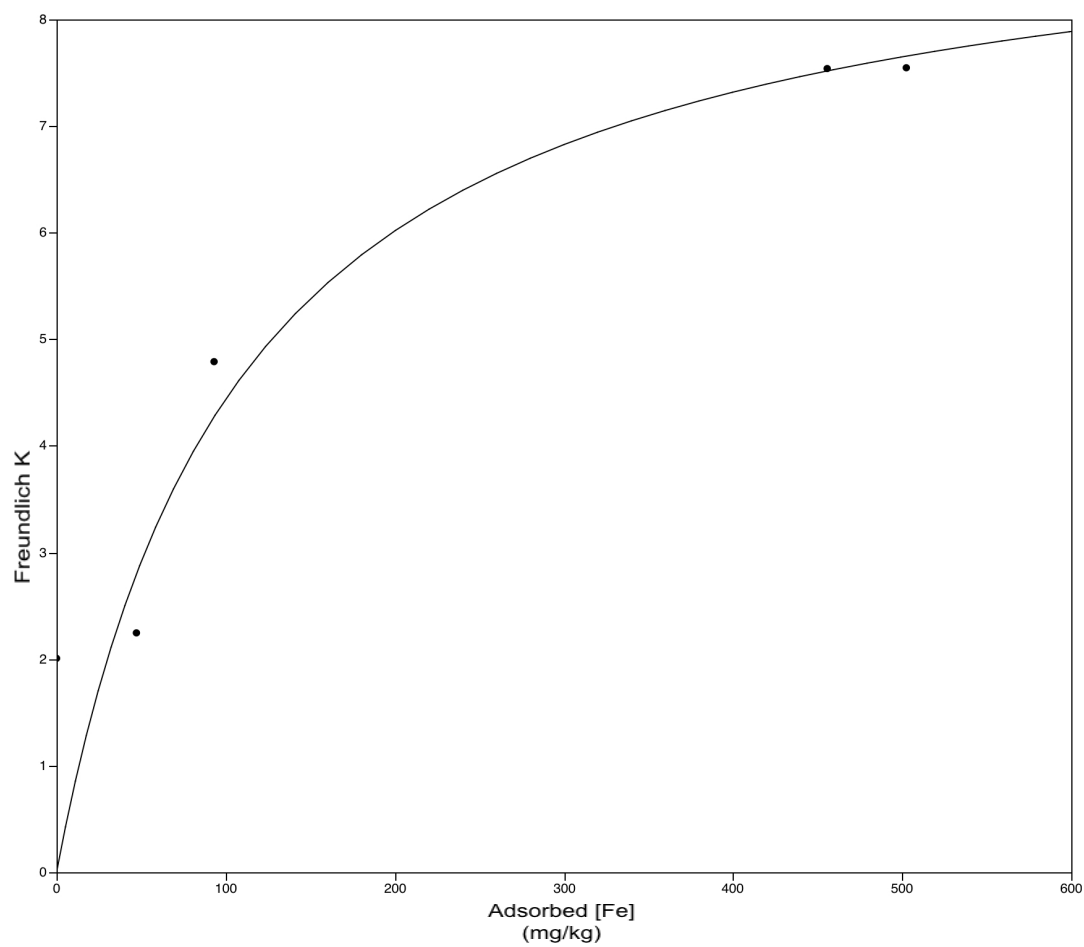


Figure 4.3 - Freundlich adsorption constant as a function of Soil Fe treatment, fitted with a Langmuir function. $R^2 = 0.8658$

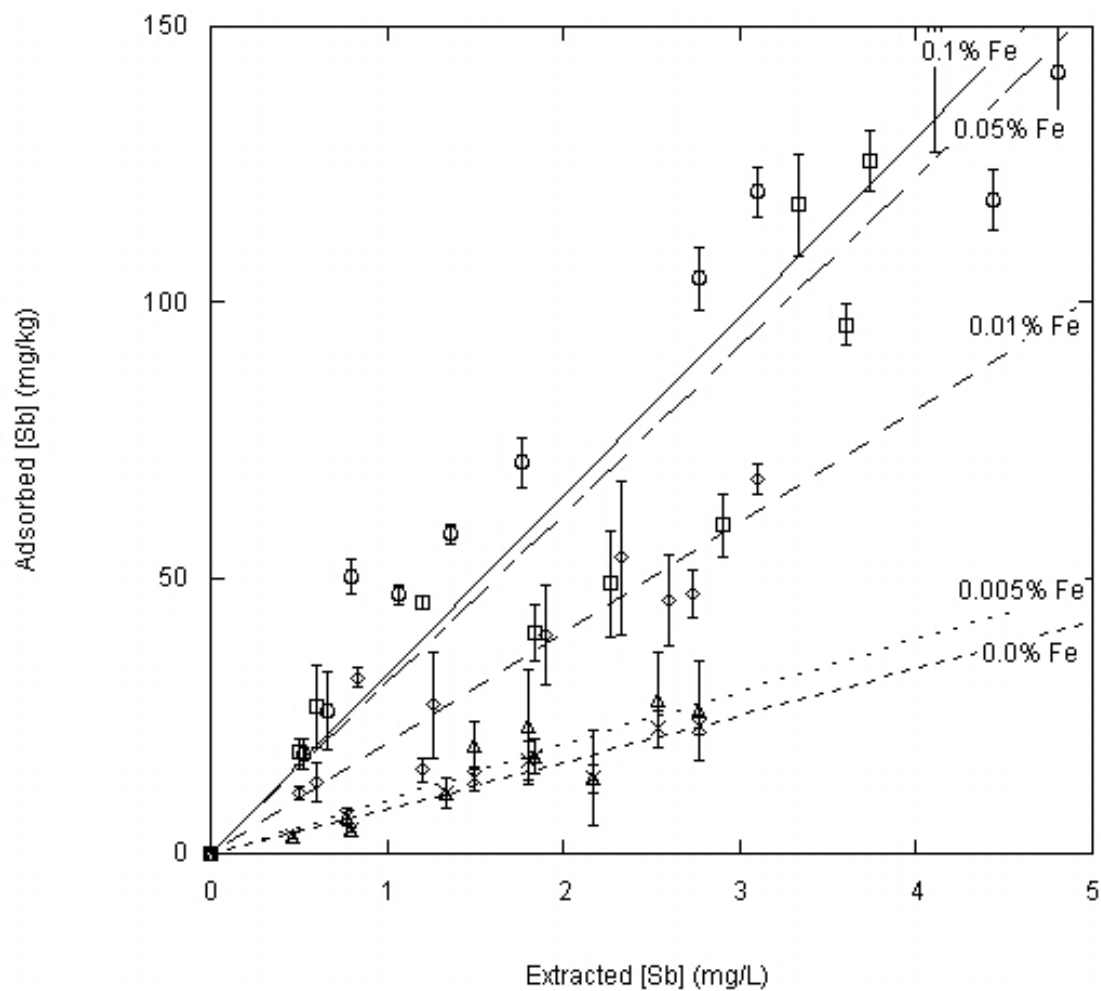


Figure 4.4 – Partition curves for Fe treated soils extracted with 1M NH_4NO_3

4.3.3.2 Phosphate Extraction

As can be seen in Figure 4.5, the partition curves for 5 soil treatments following phosphate extraction have a greater slope as the iron amendment increased from 0% to 0.05%. The change in the partition coefficient between controls and the .005% Fe treatment was minimal. The mean partition coefficient for the .01%Fe treatment was noticeably greater than for the control and .005% Fe treatment. The largest jump in the partition coefficient was observed between the .01% and the .05% Fe treatments. Lastly, K_d values decreased between 0.05% and 0.1% Fe.

4.3.3.3 Oxalate

Figure 4.6 shows the partition curves from the oxalate treatment. A wide variation was observed in partition coefficients but a clear decrease was evident across the entire range of Fe treatments (Table 4.4).

4.3.3.4 Pyrophosphate

Figure 4.7 shows the partition curves for the pyrophosphate extraction. Partition coefficients for the pyrophosphate extraction showed an opposite trend from NH_4NO_3 and phosphate extraction, with the greatest affinity of Sb for the solid phase being observed in control soils with no Fe added. Partition coefficients decreased with increasing Fe concentration until 0.05% Fe. However, the highest Fe treatment showed a larger K_d value than the 0.05%.

4.4 Discussion

4.4.1 Sb Retention

K values from the Freundlich equation represent the capacity of the adsorbent for the adsorbate and thus K values can be used to indicate differences in Sb sorption

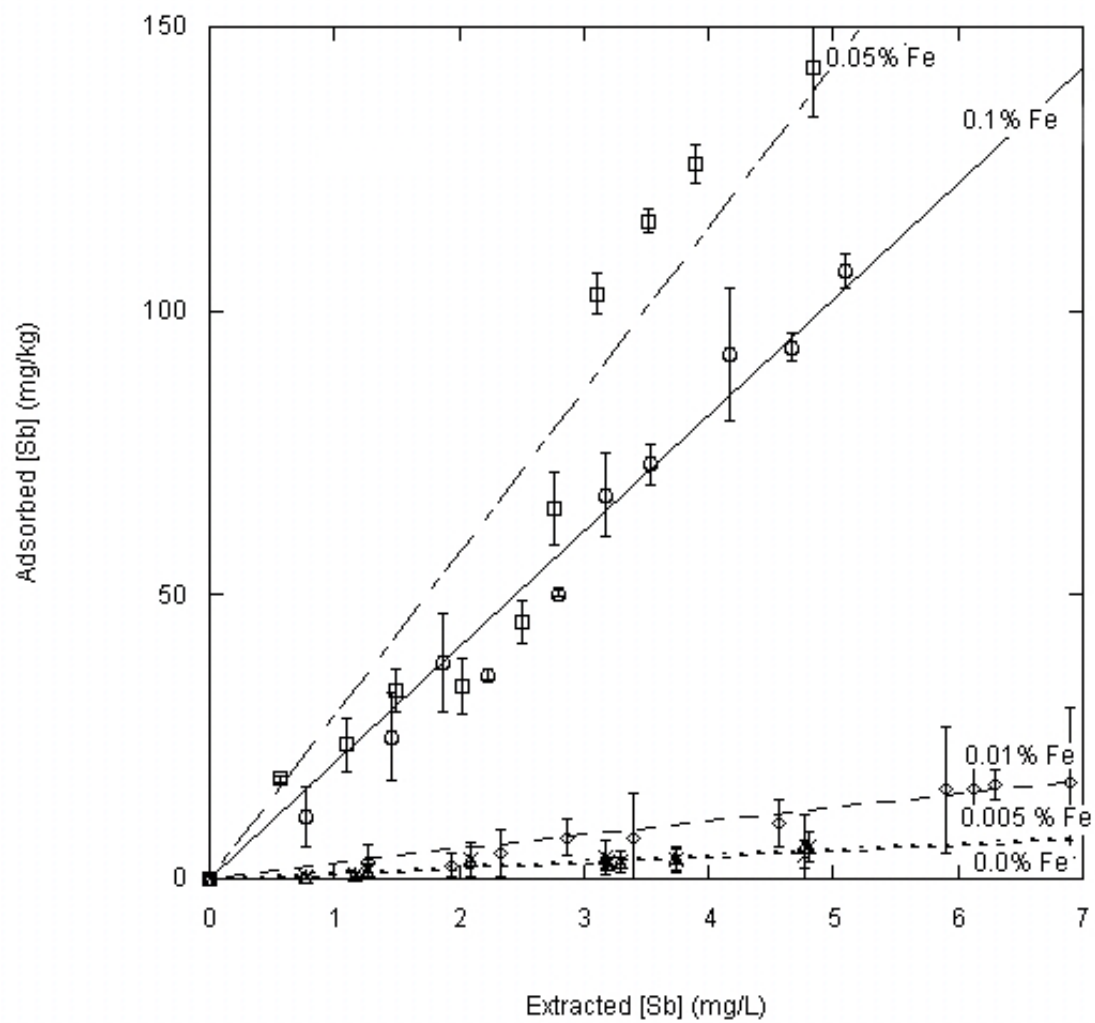


Figure 4.5 - Partition curves for Fe treated soils extracted with 0.1M Potassium Phosphate

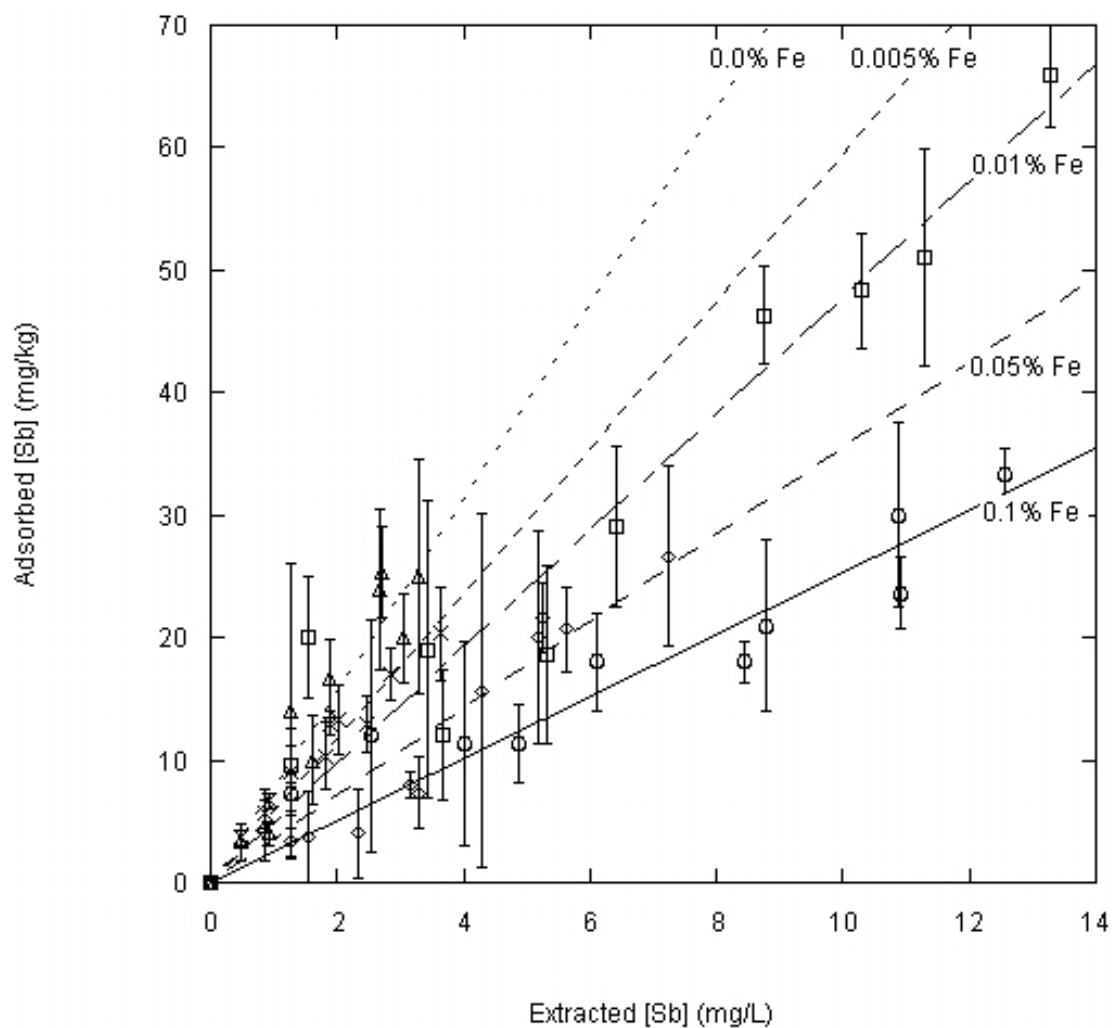


Figure 4.6 - Partition curves for Fe treated soils extracted with 0.2M Ammonium Oxalate

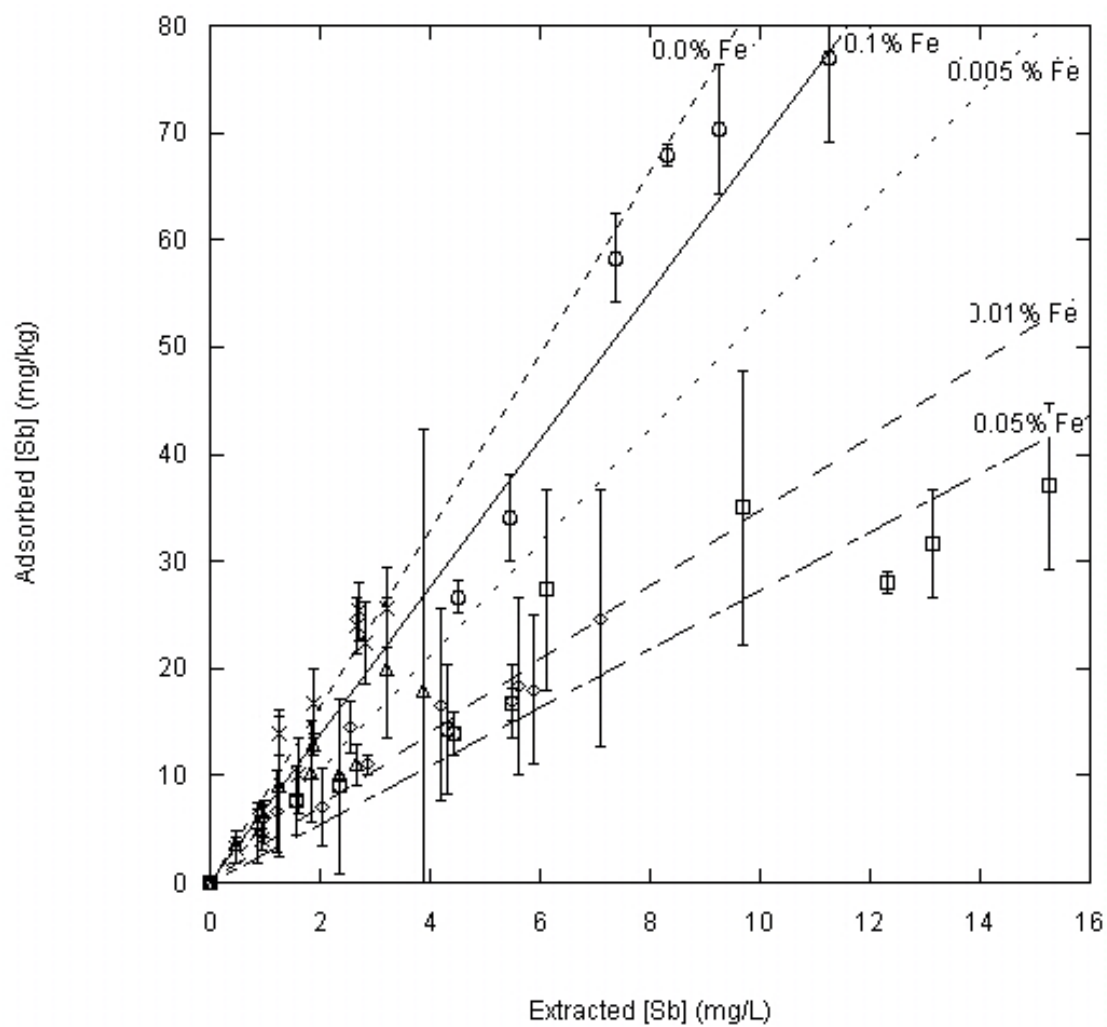


Figure 4.7 - Partition curves for Fe treated soils extracted with 0.1M Potassium Pyrophosphate

of a single adsorbent between soils (Mohan and Singh 2002). Statistical testing showed a clear and significant increase in sorption capacity, as indicated by the Freundlich sorption constants (K), of soils upon the addition of Fe above 0.005% of total soil, but sorption capacity shows a leveling effect above .05% Fe (Figure 4.3). This iron treatment range corresponds to the range in which formation of colloidal iron-organic species was observed. At 0.005% Fe no colloid formation was observed and the supernatants collected for the soil treatment contained appreciable amounts of dissolved organic matter. Above 0.1% Fe, precipitation of oxides was noticeable. In between these treatments, organic colloid formation upon iron addition was substantial. The leveling effect of sorption capacity also corresponds to the retention maximum observed for Fe during soil treatment, with saturation being reached between .05% and 0.1% Fe (Figure 4.1 and 4.3). The fact that iron retention reaches saturation at the same treatment level as Sb retention, which also corresponds to the range of organic colloid formation, suggests that formation of organic Fe species results in increased retention of Sb. n values from the Freundlich isotherms indicate the intensity of the interaction between adsorbent and adsorbate (Mohan and Singh 2002). Lack of significant differences between n values as soil Fe amendment increased indicate that adsorption strength of Sb remained the same regardless of Fe treatment. Increase in sorption capacity, while strength of adsorption remained the same could potentially signify that the binding mechanisms are remaining the same, but an increase in sorption sites may result from increasing Fe amendment.

It is possible the resulting increase in sorption capacity is directly related to the increase in organically complexed iron, suggesting a metal bridging complex. The

organic Fe complexes illustrated in chapter 3 have been shown to be important in retention of arsenate Mikutta and Kretzschmar, 2011 o the proposed structures. Leuz et al., (2006b) suggest a similar mechanism for Sb and As binding by the formation of bidentate, inner sphere corner sharing complexes to goethite. The clustered Fe structures reported in chapter 3 have a greater number of available corner sites. Thus, an increased proportion of these Fe-trimer organic complexes would provide additional adsorption sites for Sb, when compared to monomeric Fe complexes. Thus, the observed increase in Sb sorption capacity could be a result of increased Fe clusters formation as Fe treatment increased.

However, humic acid interaction with Fe in soils has been found to increase at lower pHs relating to a large number of carboxylic acid functional groups (Nambu and Yonebayashi, 1999). This agrees with previous work showing organically complexed Fe species are composed of a greater percentage of carboxylic acid-Fe interactions (Chapter 3), and further suggests that an increase in organically complexed Fe increases retention of more polar organic phases through complexation of carboxylic acid functional groups. In this experiment, low iron treatments did not result in coagulation of organic colloids (0 and .005% Fe treatments). With supernatants being removed during centrifugation, the more polar organic phases were thus removed when no coagulation occurred. Thus, the retention of these water soluble forms of organic matter via coagulation as soil Fe increased could account for the increase in Sb retention with increasing Fe.

4.4.2 Sb Mobility

The K_d parameter is a factor related to the partitioning of a contaminant

between the solid and aqueous phases at equilibrium and thus is the most common measure used to describe the extent to which contaminants are sorbed to soils in a given aqueous/solid system. The slope of the partition curve was used to determine the K_d parameter thus an increase in K_d indicates a greater proportion of total Sb associated with the solid phase.

Partition coefficients for NH_4NO_3 extractions showed a clear increase as Fe treatments increased. This indicates that the dissolved Sb(V) fraction decreases with increasing organic Fe concentration. This is consistent with the low mobile fractions (< 50% of total Sb) observed in organic soils by Tighe et al., (2005b) but does not elucidate whether iron-organic complexes are responsible for the increased retention, or whether the increased concentration of the more polar organic fraction in the solid phase at higher Fe results in increasing Sb retention.

A similar trend was observed for partition coefficients during phosphate extractions, with proportion of Sb associated with the solid phases being greater at higher organic Fe levels versus low Fe treatments. However, a significant decrease in partition coefficient was observed between the 0.05% Fe amendment and the 0.1% Fe amendment. Considering that the organic fractions are over saturated with respect to Fe at this Fe level, a large variation in the Fe content of these treated soils is likely (Figure 3.1) and may account for the observed decrease at the highest Fe treatment.

Considering phosphate's strong affinity for Fe it seems unlikely that Sb bound to organically complexed Fe would not be displaced by phosphate extraction. However, additional Fe amendments could be providing additional adsorption for phosphate as well as antimonate. Thus, the decrease in phosphate extractable Sb at

higher soil Fe additions could be a result of an abundance of binding sites, which would eliminate the competition between Sb and phosphate.

Oxalate and pyrophosphate are common extractants of soil Fe. The inclusion of these extractions in the soil mobility study was intended as a link between the iron content of the soil and Sb mobility. As the Fe amendment increased, the amount of Sb released by oxalate extraction also increased, as indicated by decreasing partition coefficients (Table 3.4). Dissolution, the mechanism whereby organic anions change the chemical structure of the surface bound species through metal complexation and removal, could account for the change in Sb sorption by oxalate. The relationship between chelation, dissolution and sorption are affected by preferential removal of Fe and Al from the sorbing surface, thus decreasing the number or strength of sites available for these sites to sorb P, and perhaps Sb (Guppy et al., 2005). Oxalate was shown to effectively prevent sorption of available P as well as solubilize bound P (Staunton and LePrince, 1996). Release of native P and reduction of P sorption were both strongly correlated with release of polyvalent cations, such as aluminum, during extraction with other organic acids (Swenson et al., 1949; Ohno and Crannell 1996, Fox and Comerford, 1992). However, if dissolution of Fe and Al is the primary mechanism by which Sb sorption capacity is reduced, then organic ligands must actively complex and solubilize metals associated with Sb, a mechanism which has not been directly examined (Guppy et al., 2005). Oxalate has also been shown to form stable complexes with Sb. Thus, it is unclear whether oxalate was extracting Fe and releasing any Sb associated with it, or simply extracting Sb directly, independently of Fe extraction.

Pyrophosphate partition coefficients reflected the removal of organically complexed Fe. Thus, Sb released by these extractions potentially represents Sb bound by metal bridging complexes. Pyrophosphate would have been expected to show similar results to oxalate if dissolution of iron complexes were the dominant mechanism of Sb release. For the most part a similar trend was observed in partition coefficients was observed between oxalate and pyrophosphate (Table 3.4). A decrease in partition coefficients was observed as Fe treatment increased from 0 – 0.05% indicating an increase in the amount of Sb retained. However, the highest Fe treatment does not follow the same trend as the 0 – 0.05% Fe treatments, showing a larger than expected distribution coefficient. The high Fe soil treatment in the phosphate extraction showed a similar deviation from the trend observed the the soils with lower Fe. This represents a shift in the binding patterns of antimony at higher Fe loadings.

Structural examination of Fe-organic complexes may potentially account for the observed change in Sb binding in pyrophosphate and phosphate extractions. The proportions of the two Fe sites proposed in Chapter 3 may be changing as Fe loadings increased. Increasing the number of trimer sites relative to the number of monomeric iron complexes would provide more adsorption sites for phosphate, resulting in the decline in Sb extracted. Similarly, if pyrophosphate preferentially dissolves monomeric Fe over Fe trimers then pyrophosphate would extract less Fe as the number of these trimer complexes increases relative to the number of monomeric Fe sites.

These extractions indicate that common extractants for Fe are capable of

mobilizing Sb in organic soils, suggesting that Sb retention in these soils is related to the Fe concentration. Considering significant portions of soil Sb were not exchangeable by either phosphate or NH_4NO_3 suggests, it can be concluded that simple anion exchange mechanisms do not explain Sb retention. Thus, Sb retention must be a more specific chemisorption reaction. The mobilization of Sb upon addition of oxalate could have implications for the bioavailability of Sb, considering the abundance of low molecular weight organic acids excreted into the rhizosphere by plants.

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CHAPTER 5

INFLUENCE OF ORGANICALLY COMPLEXED IRON AND ORGANIC ACIDS ON BIOAVAILABILITY OF ANTIMONY TO MAIZE SEEDLINGS IN ORGANIC SOILS

5.1 Introduction

Antimony (Sb) is listed as a suspected human carcinogen by the U.S EPA and the toxicity of Sb to humans and animals is considered comparable to its sister element arsenic (Belzile et al., 2001; Bowen 1979). Natural Sb background concentrations in soil were found to vary between 0.3 - 8.6 mg kg⁻¹ (Johnson et al. 2005; Kabata-Pendias and Pendias 1984). However, use of Sb as a component of many industrial products, e.g. in fire retardants, brakes, semiconductors, plastics and metal alloys (Filella et al. 2002a; Mathys et al. 2007), has increased emission through human activities into the environment. High soil Sb concentrations are often found in areas affected by Sb-mining activities (210 to 360 mg kg⁻¹ in Ainsworth et al. (1990); 192 to 15,112 mg kg⁻¹ in Baroni et al. (2000); 31 to 5,986 mg kg⁻¹ in Pratas et al. (2005)), and along roadsides (1.2 to 8.7 mg kg⁻¹ in Amereih et al. (2005))

Previous studies have shown that plants show elevated tissue Sb concentrations when grown in Sb contaminated soil. Foliar Sb concentrations of up to 1100 mg kg⁻¹ were measured in vegetation growing on a soil polluted with up to 400 mg kg⁻¹ Sb dry weight (DW) in the vicinity of an Sb smelter in north-east England (Ainsworth et al. 1990). Another study reported foliar Sb concentrations greater than 100 mg kg⁻¹ in plants growing on a mine tailing soil with 9000 mg kg⁻¹ Sb DW. More than 1000 mg

kg⁻¹ Sb were found in the basal leaves of *Achillea ageratum* grown on a site contaminated by mining (Baroni et al. 2000). There are also studies that reported only small Sb concentrations in plants grown on heavily Sb-contaminated soils. Pratas et al. (2005) reported maximum stem concentrations of less than 5 mg kg⁻¹ Sb DW in various tree and herb species growing on a Portuguese mine spoil with an average total Sb concentration of 663 mg kg⁻¹. In one study, barley (*Hordeum vulgare* L.), grown in sand contaminated with 100 mg kg⁻¹ soluble Sb was not found to take up more than 2 mg kg⁻¹ Sb DW, although yields were reduced (Davis et al., 1978). In another study, 19 species of garden and crop plants were grown on potted soil that had been spiked with Sb to give a dissolved Sb concentration of 45 mg L⁻¹. The plants accumulated up to 399 mg kg⁻¹ Sb DW in the shoots without showing toxicity symptoms (Hammel et al., 2000).

Previous work in this thesis has examined the role metal bridging complexes to natural organic matter have on Sb mobility. It was found that the more organically complexed Fe present in an organic soil, the less mobile Sb becomes (Chapter 4). These results suggest that organic-iron complexes could play a significant role in Sb mobility and could decrease the bioavailability of Sb.

The release of organic acids from roots has also been implicated as a mechanism to enhance the availability of sparingly soluble soil nutrients such as P, Zn, Fe and Cu in the rhizosphere (Marschner, 1995; Jones et al., 1996; Barber, 1995; Jones, 1998; Tinker and Nye, 2000). It has become almost universally accepted that organic acids cause a significant mobilization of P in the rhizosphere (Marschner, 1995). Malate and citrate appear to be the primary components released by roots

under P deficiency (Jones, 1998). Organic acids such as citrate and malate are also potent complexers of Fe in soil and induce the dissolution of previously unavailable insoluble ferric oxyhydroxides (Gerke, 1992; Jones et al., 1996). Recently it has been proposed that citrate may play an important role in supplying Fe to dicotyledonous plants (Jones et al., 1996a). Fe deficiency induces a substantial accumulation of organic acids in root tissues and also induces a large (5-10 fold) increase in organic acid excretion (De Vos et al., 1986; Yi and Guerinot, 1996; Landsburg, 1981; Ohwaki and Sugahara, 1997). Under Fe deficiency, root H⁺-ATPases are highly up-regulated, resulting in an increase in excreted H⁺ (Rabotti and Zocchi, 1994). In calcareous soils, the combination of the acidification potential in addition to the complexing ability of citrate, which increases with decreasing pH, provide a viable means of mobilizing Fe in the rhizosphere. Once Fe (III)-citrate complexes form in the soil solution, dicotyledonous roots can access the Fe using a plasma-membrane ferric reductase which reduces Fe(III) to Fe (II), releasing the citrate back into solution.

Extraction of Fe treated organic soils with oxalate showed a significant dissolution of Sb (Chapter 3). Thus, organic acids released into the rhizosphere could potentially produce more soluble Sb species and, as was shown by Hammel et al., (2000) and Tschan et al. (2009), soluble Sb correlates strongly with plant uptake. Thus, the bioavailability of Sb needs to be expressly examined in relation to these complexes and potential effects from plant exudates, particularly organic acids.

Organic soils, treated with Fe in the range to produce organic-Fe complexes, were spiked with Sb(V) and used to examine the effect organic-Fe complexes have on bioavailability of Sb to maize seedlings. Additionally the role of organic acids in

mobilizing Sb was examined by extracting treated soils with model organic acids that are common to the maize rhizosphere.

5.2 Materials and Methods

5.2.1 Soil Treatment

Organic rich soils were collected from a farm in New Hampton, New York. All soils were sampled at random along an uncultivated 50m transect. Samples were taken between 0 – 18 inches below the soil surface. Soils were returned to the lab, sieved to < 2mm and dried before treatment with iron. pH measurements for each soil were taken using 1M CaCl₂. Soil physical and chemical properties are reported in Table 5.1.

Table 5.1 Chemical Characteristics of collected soils before iron-treatment. Parenthesis represents standard deviation of triplicate samples.

| pH | Organic Carbon (%) | CEC (meq kg ⁻¹) | Free Fe Oxides (mg kg ⁻¹) |
|------------|--------------------|-----------------------------|---------------------------------------|
| 5.48 (.26) | 37.4 (3.47) | 594 (73.7) | 4430 (657) |

Iron treatment of soils was performed in bulk batches; 20kg of sieved, dried soils were weighed out into acid washed, 25 L polyethylene containers. 20L of a .01M potassium nitrate (KNO₃) solution was added to create a 1:1 (m/v) soil suspension. The suspension was stirred vigorously for 5 minutes and then allowed to rest for 30 minutes before addition of iron. Ferric iron was added to the suspension, as iron chloride (FeCl₃·6H₂O), to reach 0, 0.01, 0.05 and 0.1% Fe in the soil, by mass. Suspensions were stirred vigorously for 30 minutes. Treated soils were allowed to air dry and equilibrate at 25°C for four weeks.

5.2.2 Pot Experiment

1 kg of treated soils was placed in 2 L pots. Pots were sealed to create a closed system with respect to Sb. Sb(V) was added as solid potassium antimonate ($\text{KSb}(\text{OH})_6$) to attain a soil Sb concentrations of 50, 100, 200, 400, 800 and 1600 mg kg^{-1} . Control pots received no Sb treatment. Pots were allowed to equilibrate for 2 weeks under greenhouse conditions with regular watering. Soil treatments were duplicated for each Sb treatment at each soil Fe level. Maize seeds were germinated for 72 hours in sterile water-saturated vermiculite. Four maize seedlings were transplanted to each pot. Maize plants were grown under greenhouse conditions on a 12 hour light cycle, and watered daily until harvest at four weeks.

5.2.2.1 Sample preparation

Plants were removed from the soil and rinsed with deionized water, then separated into roots and shoots and oven-dried for 48 h at 65°C. The dry weight of each root and shoot sample was recorded before digestion. Samples were ground in a mortar and pestle and 0.2g of each sample was weighed out into analytically clean digestion tubes. 5ml of concentrated nitric acid was added to each digest and left for 12 hours at room temperature. Digestion tubes were then transferred to a heating block and brought to 65°C for 3 minutes. 3mL of hydrogen peroxide (30%) was added to each digest. When effervescence had ceased an additional 3 mL of H_2O_2 was added. This process was repeated until no effervescence was observed upon addition of H_2O_2 . Digests were then allowed to reduce to approximately 3 mL before being diluted to 50mL with deionized water. These plant tissue extracts were saved for Sb and Fe analysis.

Total soil Sb was determined using nitric acid digestion of soils sampled after

harvest. The digestion involved adding 1g of soil to a glass tube containing 2.5 mL concentrated HNO_3 and 10 mL conc. HCl . Samples were placed in a heating block and heated to reflux for 15 minutes. A watch glass was used to cover refluxing samples for vapor recovery. Digests were filtered through Whatman No. 42 filter paper and filtrates were collected in a 100mL volumetric flask. Filter papers were washed with 5 mL 95°C HCl , and 20 mL of deionized water. Washings were collected in the same 100-mL volumetric flask and brought up to 100mL with deionized water.

5.2.2.2 Sample Analysis

Sb concentrations of plant and soil digests were determined using flame atomic absorption spectroscopy at a wavelength of 206.8nm. Digests below the detection range were further examined using standard additions. The mean plant tissue concentration was calculated in mg kg^{-1} dry weight. Bioaccumulation factors (BAF) were calculated as the ratio of concentrations in the plant to the environmental concentration at equilibrium. BAFs were determined by linear modeling of tissue Sb concentrations regressed against soil Sb concentrations

5.2.3 Organic Acid Extraction

Determination of the mobilizing affects of organic acids from maize roots was conducted using soils taken from pot experiments before planting of maize seedling. From the 800 mg kg^{-1} Sb treatment, 5g of soil was sampled from each Fe treatment level and shaken with an organic acid solution. 0.5mM solutions of citrate, oxalate, malate and glutamate were made using the sodium salts of these ligands. NH_4NO_3 has been used in the previous work to assess bioavailable Sb (Nakamaru et al., 2006) and

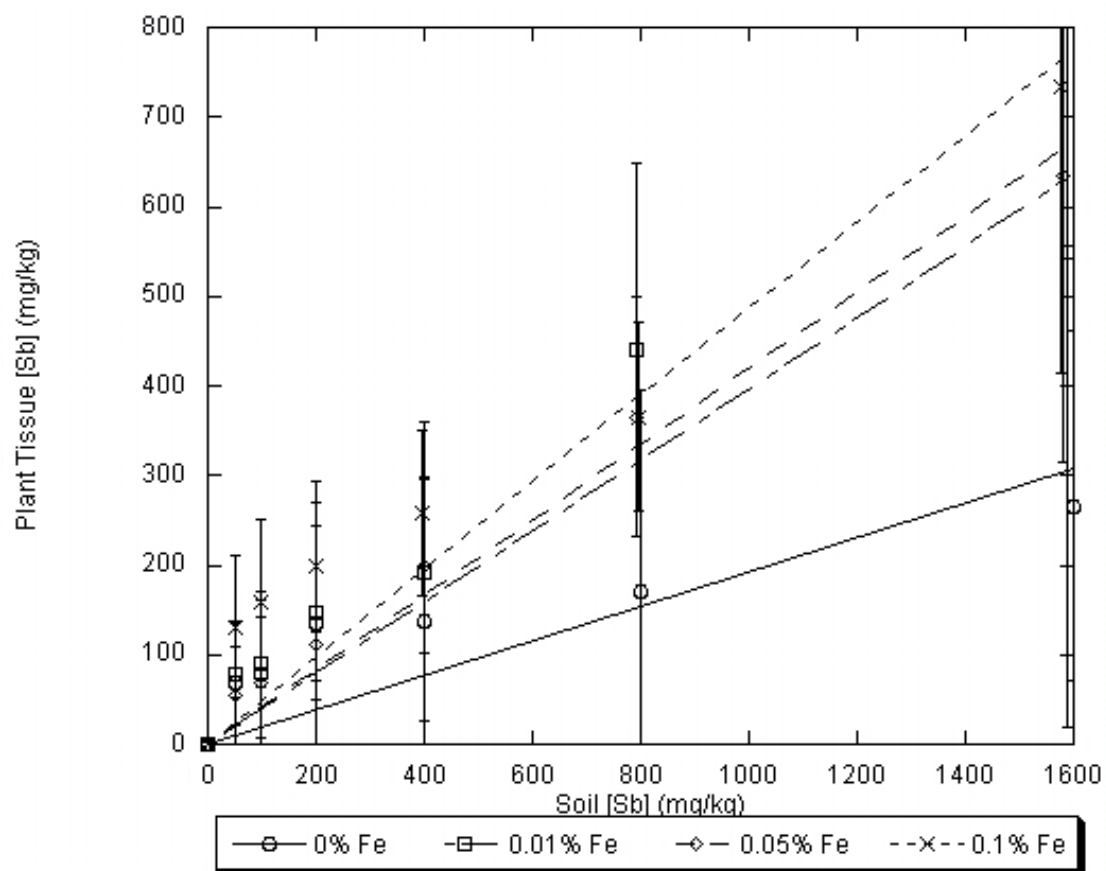


Figure 5.1 – Plant tissue Sb concentration as a function of soil Sb concentration
 R^2 0% - 0.845 ; 0.01% - 0.923 ; 0.05% - .894 ; 0.1% - .964

was used as a control extraction. 50mL of each solution was shaken with 5g of soil for 12 hours. Samples were then centrifuged and filtered through Whatman 42 filter papers. Filtrates were analyzed for Sb concentration using flame AA at 206.8nm and for Fe using flame AA at 248.3nm.

5.3 Results

5.3.1 Sb accumulation

Maize dry weight was not correlated with Sb tissue concentration in any of the Fe-treated soils (Pearson's correlation), indicating that Sb was not phytotoxic in the concentration range tested. Plant uptake of Sb in Fe treated soils is shown in Figure 5.1. In all soil treatments plant tissue Sb was strongly correlated with soil Sb (Figure 5.1). The resulting bioaccumulation factors, calculated from the slopes of the best fit lines in Figure 5.1 are shown in Table 5.2. All Fe-amended soils show significantly greater Sb concentrations in the maize crop in absolute concentration terms, which is demonstrated by the statistically significant differences between bioaccumulation factors (BAFs) of the 0.0% Fe soil and all Fe treated soils (Kruskal Wallis $P < .001$). No significant differences were observed between BAF for the 0.01% Fe treatment and all other Fe treated soils ($P = .600$, $P = 0.172$), likely due to the high variance observed for the 0.01% treatment. The 0.1% Fe treatment was found to have a significantly higher BAF overall, compared to the 0.05% Fe treatment ($P = .0357$)

Table 5.2 – Sb Bioaccumulation Factors for Maize Grown in Fe treated soils. was used as a control extraction. 50mL of each solution was shaken with 5g of soil for
Parentheses represent standard deviation of 8 replicate samples

| Soil Fe Treatment | 0.0% | 0.01% | 0.05% | 0.1% |
|------------------------------|------------------|-------------------|-----------------|------------------|
| Bioaccumulation factor (BAF) | 0.191 (0.051) | 0.399 (0.125) | 0.421 (0.07) | 0.485 (0.079) |

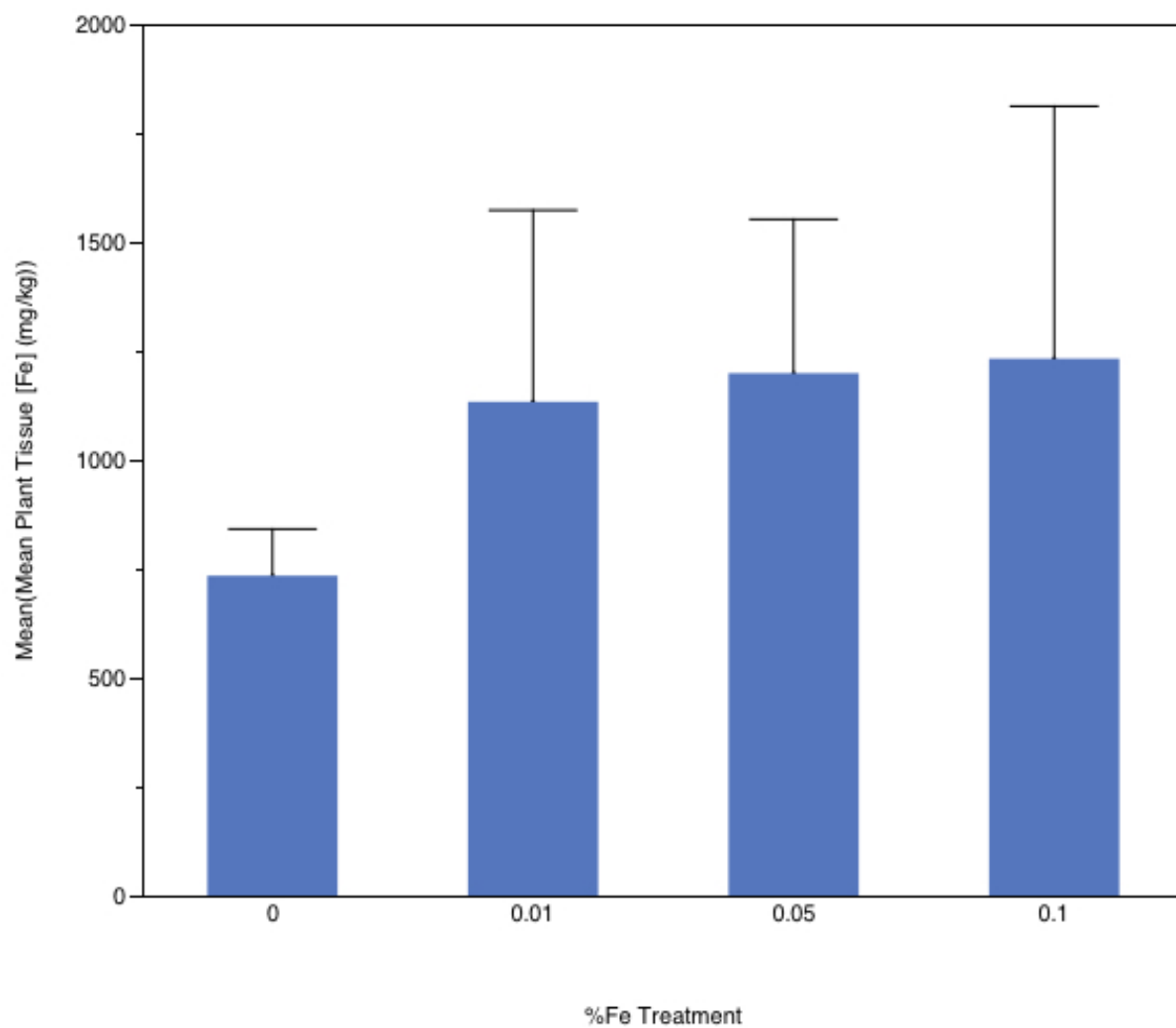


Figure 5.2 - Mean plant tissue iron concentration in soils treated with Fe at 0, 0.01, 0.05 and 0.1%

Mean Fe concentrations in maize tissue for each soil treatment are presented in Figure 5.2. All seedlings grown in soils treated with Fe had significantly greater Fe tissue concentrations than the 0% Fe treatment (ANOVA $P < .001$). Differences in maize tissue Fe for all iron treated soils were not statistically significant (ANOVA, $P = 0.522$).

5.3.2 Organic Acid Extraction

Amounts of Sb extracted per kg of soil, by the organic acids are shown in Figure 5.3. In the NH_4NO_3 extraction, Sb extracted from the 0% Fe treatment was significantly higher than Sb extracted from all Fe treated soils (Kruskal Wallis, $P = .0046$). Glutamate extractions showed no significant differences in extracted Sb between control soils and the 0.01% and 0.1% Fe treated soils (Kruskal-Wallis $P = 0.1959$). Sb extracted from the 0.05% Fe treatment was significantly higher than all other soils ($P = 0.002$).

Malate extracted significantly greater Sb concentrations from the Fe treated soils than from controls ($P < 0.001$). No significant differences were observed between the 0.01% and 0.05% treatments ($P = 0.359$), whereas the 0.1% Fe treatment showed significantly greater extracted Sb than all other Fe soil treatments ($P = 0.024$).

Oxalate also extracted significantly greater concentrations of Sb as Fe amendment of the soil increased. Extracted Sb in all Fe treated soils was statistically greater than 0.0% Fe soils ($P < 0.001$). Although no significant difference was observed between the 0.01% and 0.05% Fe treatments, the Sb concentrations from extraction of the 0.1% Fe soil were significantly greater than in all other Fe treated soils ($P = 0.002$). The same trend was observed for citrate extractions, with

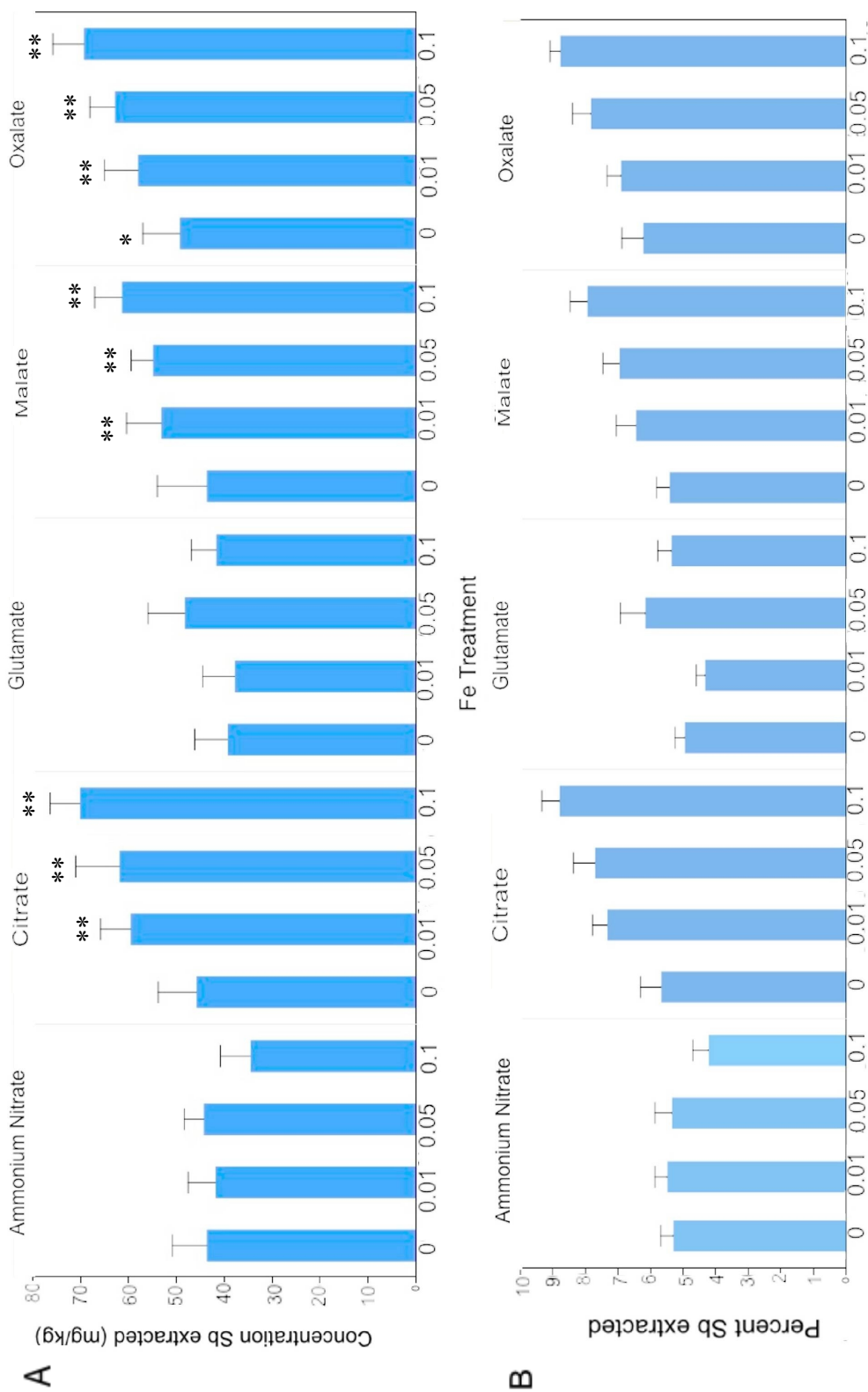


Figure 5.3 - (A) Sb extracted (mg/kg) from iron treated soils by model organic acids and ammonium nitrate. (B) Percent Sb extracted from iron treated soils by organic acid and ammonium nitrate treatment
 * indicates statistically significant difference from corresponding NH_4NO_3 extraction at $P < .05$ ** $P < .01$.

significantly more Sb extracted from all Fe treated soils compared to soils that did not receive Fe treatment ($P < 0.001$). Similarly, increases in extracted Sb were significantly greater for the 0.1% Fe treated soil when compared to lower Fe soil treatments ($P = 0.041$). A significant increase in Sb extracted was also observed in the 0.05% Fe soil compared to the 0.1% soil ($P = 0.0257$).

When compared to the NH_4NO_3 extracted Sb fraction, malate, citrate and oxalate solubilized significantly more Sb at all Fe treatments, but not in the control soils which received no Fe treatment. Oxalate was the only extractant that dissolved significantly more Sb than NH_4NO_3 extraction in the unamended soils. Glutamate did not significantly increase Sb extraction at any of the Fe treatments, when compared to the NH_4NO_3 extraction (Figure 5.3).

The percent of total soil Sb extracted by each of the organic acids, and ammonium nitrate is shown in Figure 5.3a. Ammonium nitrate extracted a significantly smaller percentage of total soil Sb in the highest Fe treatment ($P = .032$). No trends were observed in the percent of total soil Sb extracted by glutamate as Fe treatment increased. The malate, oxalate and citrate extractions solubilized a greater percentage of the total soil Sb as the Fe treatment level increased ($P < .001$).

Fe extracted per kg soil during treatment with the organic acids is shown in Figure 5.4. In the NH_4NO_3 extraction, no significant difference in Fe extracted was found between any of the soils examined. Similarly, glutamate showed no significant differences in extracted Fe for soils treated with varying levels of Fe. Malate extracted significantly greater concentrations of Fe in the Fe-treated soils than controls ($P < 0.001$). A significant increase in Fe extracted was observed as the soil Fe level

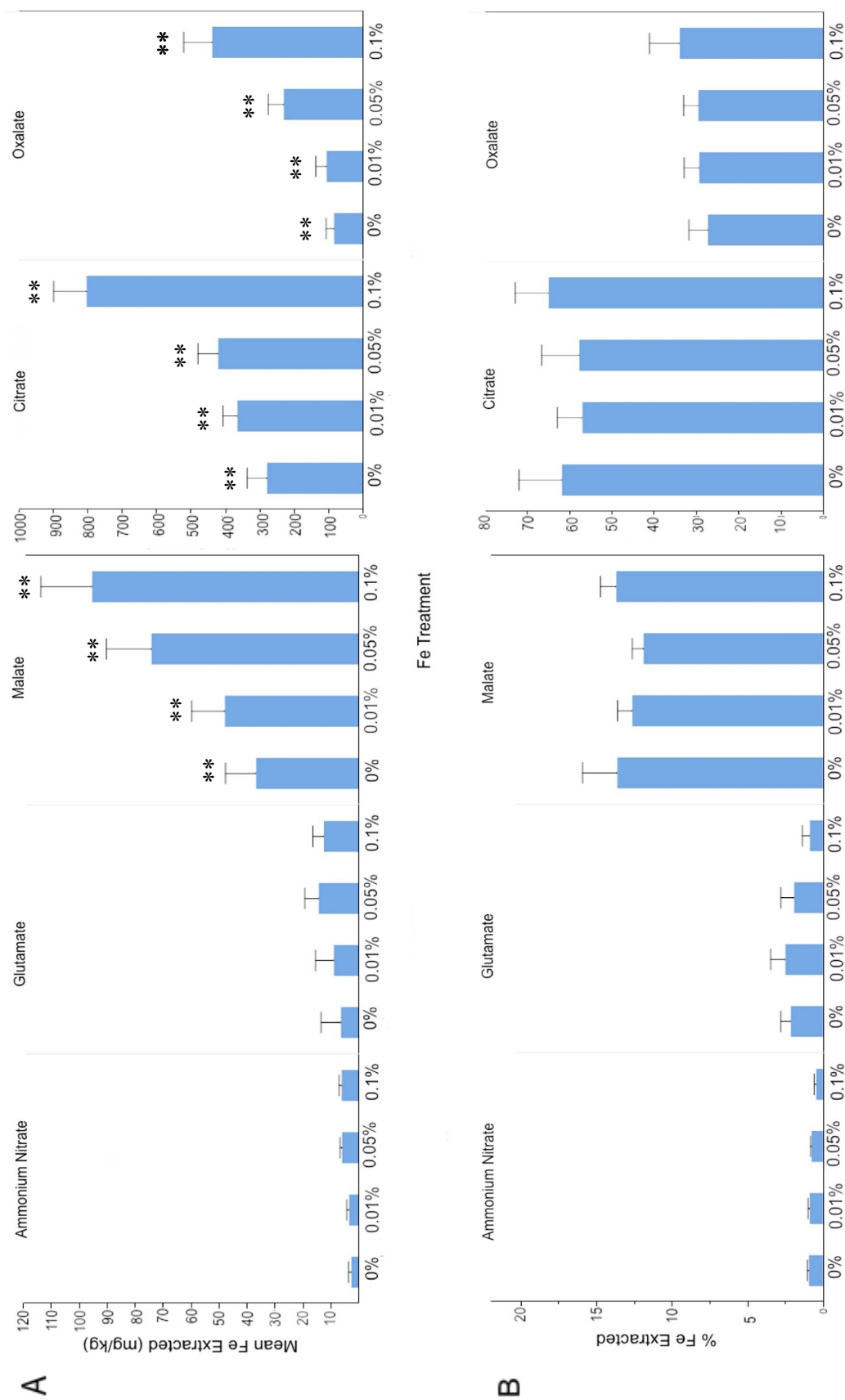


Figure 5.4 – Iron concentrations extracted during treatment with model organic acids, oxalate and citrate are included separately for scale (B) Percent Fe extracted during organic acid treatment
**** indicates statistical significance from the corresponding NH_4NO_3 extraction at $P < .01$**

increased ($P < .001$).

Oxalate also extracted significantly greater Fe as Fe treatment increased ($P < .001$). Extracted Fe was statistically greater for all Fe-treated soils than the 0% Fe-treated soils ($P < .001$). The same trend was observed for citrate extractions, with significant increases in Fe extracted for all Fe-treated soils relative to the 0% Fe controls. Similarly, as Fe treatment level increased, significantly higher Fe was extracted ($P < .001$).

When compared to the NH_4NO_3 extraction, malate, citrate and oxalate caused significantly greater Fe dissolution at all soil Fe treatment levels. Glutamate again did not show significantly increased Fe extractions in any of the soils when compared to the NH_4NO_3 extraction (Figure 5.4).

Figure 5.4b shows the percent of total soil Fe extracted by each treatment. Ammonium nitrate extracted significantly less Fe, as a percent of total, in the highest Fe treatment compared to the sample without Fe treatment ($P = .024$). This downward trend in Fe extraction was observed in the glutamate extraction as well ($P = .009$). No significant changes were observed in the percentage of Fe extracted by malate, citrate or oxalate as Fe treatment increases. In terms of overall extraction efficiency, citrate extracted a significantly greater percentage of total soil Fe in all the Fe treated soils oxalate extracted significantly more than malate, glutamate and ammonium nitrate and malate extracted a greater percentage of Fe than glutamate and ammonium nitrate.

5.4 Discussion

The calculated BAFs for maize grown in Fe-treated soil increased progressively with greater soil Fe addition. This indicates a consistently greater

phytoavailability of Sb in soils with greater Fe treatments. Considering the NH_4NO_3 extraction, indicating bioavailable Fe, showed a significant decrease in the amount of Sb extracted as Fe treatment increased, as well as the observed increases in Sb retention observed in Chapter 4 these results deviate from what was predicted at the outset of this study.

The Fe tissue concentrations in the pot experiment show increased Fe tissue levels in the higher Fe treatments. It is likely that this increase is due to the increase in Fe treatment alone. Greater Fe in the maize tissue could be a result of root exudates mobilizing a greater percentage of iron from the soil. As the organic acid extractions indicate, even at the same level of organic acid treatment significantly more Fe could be solubilized in the higher Fe treated soils. The Fe concentration observed in the maize tissue was greater in all the treated soils compared to the untreated soil, but no significant changes were observed between any of the Fe treated soils. This points to saturation in plant Fe uptake in Fe amended soils.

As soil Fe treatment increased, the total amount of Fe extracted by malate, oxalate and citrate increased. However, the percentage of total Fe extracted remained constant. This indicates that the additional iron was extracted by the organic acids resulting in the net increase of Fe extracted (mg/kg). The ammonium nitrate and glutamate extraction showed a clear decrease in the percentage of total Fe extracted suggesting that the added iron was not readily exchangeable.

Extraction with organic acids showed that soils with higher Fe released more Sb into solution. Citrate and oxalate stood out in their ability to release Sb from Fe-treated soils as well as control soils. The amount Sb extracted by malate, citrate and

oxalate increased with iron treatment suggesting a connection between Fe content of the soil and release of Sb. A greater percentage of Sb was exchangeable in the higher Fe soils, which could potentially indicate that the Sb bound by ternary complexes is easily displaced by organic acids common to the maize rhizosphere.

On a mass basis, citrate and oxalate extracted more antimony in a single extraction than was accumulated in maize plants during the pot experiment over a four week growth period. The concentrations of the organic acids used in these extractions is likely to be much higher than would be found in the rhizosphere of maize, and the amount of Sb detected in the plant tissues is likely to be the result of a lower concentration of a mixture of organic acids releasing small amounts of bioavailable Sb over time. Together, these data suggest that Sb is mobilized by organic acids common to the maize rhizosphere. Thus, NH_4NO_3 extraction may not truly represent bioavailable Sb in organic soils. Additional work must be done to determine the effect of organic acid exudation in the rhizosphere has on bioavailability of Sb in mineral soils.

The corresponding extraction of Fe and Sb by organic acids at increased soil Fe addition could point to a linkage between organically complexed Fe and Sb retention. This could indicate ternary metal bridging complexes affecting Sb mobility, but could also result from simple extraction of soil bound Fe and Sb by organic acids as independent processes. Citrate and oxalate have been shown to have significant affinities for Sb as well as Fe and could thus extract Sb and Fe simultaneously through completely separate reactions (Tella and Pokrovski, 2011).

Exudation of organic acids in the rhizosphere of maize is common during

various nutrient deficiencies (Strom et al., 2002, Jones et al., 1996). Iron deficiency results in appreciable exudation of citrate and malate (Jones et al., 1998). Maize has also been found to produce phytosiderophores in response to Fe deficiency (Bernards et al., 2002). Although, phytosiderophores are not examined in this particular study they remain of interest in Sb mobilization based on their ability to mobilize unavailable forms of Fe by a similar mechanism as the organic acids examined here. Organic acid exudation from maize roots also increased under P limiting conditions, and Fe and P uptake by plants roots has been found to be related under both P and Fe limitation (Strom et al., 2002). Regardless of the mechanism of organic acid release, the data presented in this chapter suggest that plant processes responsible for nutrient uptake in limiting conditions may have a significant effect on Sb bioavailability in organic soils. Thus, the potential impacts of nutrient limitation on the bioavailability Sb, and other anionic contaminants, requires additional attention, particularly in relation to organic soils where metal bridging complexes are likely to account for a larger portion of anionic sorption sites.

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SUMMARY

The studies described in this dissertation address the formation of iron complexes in soil organic materials and the potential implications they have on the mobility and bioavailability of antimony.

The first chapter reveals the structure of organically complexed iron. Two distinct iron sites were identified. A monomeric Fe and an oligomeric Fe site. Oligomeric Fe complexes consist primarily of trimers of Fe but do not are not large enough or crystalline enough to register as an iron oxide.

Chapter 2 describes the interactions of Sb with pure Fe-organic complexes. These organic complexes showed greater affinity for Sb than an organic soil. A notable change in Sb retention was observed in the highest Fe treatments. This chapter also describes the affect of phosphate addition on the retention of Sb. In the pure Fe-organic complexes, phosphate significantly reduced Sb sorption capacity. Chapter 3 illustrates the affect of Fe complexes on Sb retention. Increased retention of Sb was evident with increasing Fe treatment of organic soils. The bound Sb was not extractable by ammonium nitrate or potassium phosphate, which have traditionally been used to assess bioavailable Sb. Extractants commonly used to extract Fe released significantly more Sb in organic soils treated with Sb. Soils receiving the highest Fe treatment showed significantly different extraction patterns than the other Fe treated soils. The change in Sb retention and mobility behavior in materials with high iron loadings seem to be reoccurring. This is likely to be due to shifts in the structure of the Fe-organic complexes in favor of the oligomeric Fe complexes.

Chapter 4 assesses the affect of these Fe-organic complexes on the bioavailability of Sb. Considering the greater retention of Sb as Fe treatment increased, it was predicted that the plant tissue Sb concentration would be lower in maize seedlings grown in soils with greater Fe treatments. However, the bioaccumulation of Sb increased with Fe treatment. Extraction with organic soils common to the maize rhizosphere showed an increase in Sb extracted as Fe treatment increased.

Overall, these results suggest that increased Fe treatment results in increased Sb retention but Sb bound by these soils is readily solubilized by organic acids. Considering the ubiquitous release of organic acids in the rhizosphere, this translates to an increase in bioavailability despite the increased retention. This also indicates that extraction methods used to assess bioavailable Sb underestimate Sb bioavailability in organic soils.

APPENDIX

THE EFFECT OF ANTIMONY ON PHOSPHATE INTERACTIONS WITH ORGANIC IRON COMPLEXES

The effect of Sb on phosphate interactions with Fe-humic acid complexed was assessed simultaneously with the work presented in chapter 3.

Adsorptions without Sb were performed on goethite, an organic soil and our Fe-H.A complexes with increasing Fe concentrations. P solutions were made by dissolving calcium phosphate in D.I water to make solutions between 1 and 5 ppm phosphate. The Adsorptions with Sb addition were performed at Sb:P ratios of 1:1 and 2:1. Solutions were made as described in chapter 3. Phosphate concentrations of the collected solutions were determined using ICP. Phosphate concentrations below detection range for ICP were determined using the phospho-molybdate blue method.

Table A1 shows the adsorption data for phosphate on goethite. Table A2 shows sorption data for phosphate on the organic peat soil. Table A3 shows the sorption data for phosphate on pure Fe-H.A complexes.

Table A1 – Adsorption data for phosphate on goethite

| No Sb | | 1:1 Sb:P | |
|------------------------|-------------------------|------------------------|-------------------------|
| Solution [P] (mg/l) | Adsorbed [P] (mg/kg) | Solution [P] (mg/l) | Adsorbed [P] (mg/kg) |
| 0.091 | 0.004 | 0.095 | 0.2905 (.002) |
| 0.102 | 0.007 | 0.108 | 0.4892 (.007) |
| 0.077 | 0.005 | 0.378 | 0.6623 (.011) |
| 0.088 | 0.005 | 0.497 | 0.9470 (.007) |
| 0.232 | 0.003 | 0.771 | 1.223 (.007) |

Table A2 – Sorption Data for phosphate on an Organic soil

| No Sb | | 1:1 Sb:P | | 2:1 Sb:P | |
|------------------------|----------------------------|---------------------------|----------------------------|---------------------------|-------------------------|
| Solution [P] (mg/l) | Adsorbed [P] (mg/kg) | Solution [P] (mg/l) | Adsorbed [P] (mg/kg) | Solution [P] (mg/l) | Adsorbed [P] (mg/kg) |
| | | 2.560 | 0.044 | | |
| | | 4.720 | 0.028 | 4.625 | 0.038 |
| 6.265 | 0.074 | 6.676 | 0.032 | 6.865 | 0.010 |
| 9.379 | 0.062 | 9.842 | 0.008 | 9.654 | 0.035 |
| 12.706 | 0.029 | 12.692 | 0.031 | 12.395 | 0.061 |
| 14.762 | 0.03 | 14.649 | 0.035 | 14.091 | 0.09 |

Table A3 – Sorption Data for phosphate on pure Fe-HA complexes

| | No Sb | | 1:1 Sb:P | | 2:1 Sb:P | |
|------------------------------|------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|
| | Solution [P] (mg/l) | Adsorb ed [P] (mg/kg) | Solutio n [P] (mg/l) | Adsorb ed [P] (mg/kg) | Solutio n [P] (mg/l) | Adsorbed [P] (mg/kg) |
| Low Fe Complexes | 0.019 | 0.098 | 0.211 | 0.279 | 0.022 | 0.298 |
| | 0.021 | 0.198 | 0.702 | 0.430 | 0.145 | 0.486 |
| | 0.021 | 0.298 | 1.307 | 0.569 | 0.472 | 0.653 |
| | 0.262 | 0.375 | 3.447 | 0.650 | 1.491 | 0.830 |
| | 0.366 | 0.463 | | | 3.148 | 0.985 |
| | 0.375 | 0.563 | | | | |
| | 0.920 | 0.608 | | | | |
| Intermediate Fe complexes | 0.011 | 0.099 | 0.019 | 0.498 | 0.556 | 0.444 |
| | 0.011 | 0.199 | 0.236 | 0.653 | 0.442 | 0.659 |
| | 0.017 | 0.298 | 0.155 | 0.985 | 0.096 | 0.991 |
| | 0.034 | 0.397 | 0.593 | 1.241 | 0.395 | 1.261 |
| | 0.018 | 0.498 | 0.742 | 1.426 | 0.372 | 1.463 |
| | 0.027 | 0.597 | | | | |
| | 0.090 | 0.691 | | | | |

| | | | | | | |
|----------------------|-------|-------|-------|-------|-------|-------|
| High Fe complexes | 0.020 | 0.098 | 0.065 | 0.494 | 0.269 | 0.473 |
| | 0.045 | 0.196 | 0.385 | 0.662 | 0.173 | 0.683 |
| | 0.046 | 0.295 | 0.597 | 0.940 | 0.380 | 0.962 |
| | 0.047 | 0.395 | 1.649 | 1.135 | 3.278 | 0.972 |
| | 0.072 | 0.493 | 4.246 | 1.270 | 4.314 | 1.103 |
| | 0.155 | 0.585 | | | | |
| | 0.646 | 0.635 | | | | |